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ANNALS OF BOTANY

VOL. XV

171734
BOTANICAL MUSEUM

Oxford

PRINTED AT THE CLARENDON PRESS

BY HORACE HART, M.A.

PRINTER TO THE UNIVERSITY

ANNALS OF BOTANY

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VOLUME XV

London

HENRY FROWDE, M.A., AMEN CORNER, E.C.

OXFORD: CLARENDON PRESS DEPOSITORY, 116 HIGH STREET

1901

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Notes on the Conducting Tissue-System in Bryophyta.

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With Plates I and II.



INTRODUCTORY.

IT was in 1813 that A. P. de Candolle separated the groups we now call Pteridophyta and Phanerogamia under the common name of *Vasculares* from the *Cellulares* (Bryophyta and Thallophyta). Although the term 'vascular plant' (Gefässpflanze) has lost in the light of modern anatomical knowledge its literal significance as applied to plants containing vessels formed by the fusion of cells, it is still commonly and usefully applied to the Pteridophytes and Phanerogams as possessing a double conducting or 'vascular' system of well-differentiated type. In this, two definite tissue-elements—the tracheid and the sieve-tube—are the respective essential components of the two conducting tissues—the

[*Annals of Botany*, Vol. XV. No. LVII. March, 1901.]

xylem and phloem. All the members of these two highest groups of plants, except perhaps a few doubtful cases among the lower Pteridophytes, possess such a differentiated conducting system, or can clearly be shown to have lost it by degeneration.

In the Bryophyta this is not the case. Though (unlike the green Thallophyta) the great majority are land-plants, yet nearly all the Liverworts and several of the Mosses have no specialized conducting system, nor is there any reason to suppose that their ancestors ever had. But the exigencies of increasing bulk, and particularly of the erect habit, have led, in a few of the Liverworts and the majority of the Mosses, to the acquirement of a conducting system, which, while it is extremely simple in most cases, attains in the highest forms (Polytrichaceae) to a complexity not only distinctly surpassing that of the simplest true vascular plants but almost comparable with that found among highly developed Phanerogams. Now it is almost as certain as any phylogenetic thesis is likely to be, that the conducting tissues of Bryophytes have nothing directly to do with the origin of the conducting tissues of the higher plants. The main seat of the development of these tissues in Bryophytes is the gametophyte generation, which is in any case excluded from the comparison, since the vascular system in Pteridophytes is confined to the sporophyte. And at the least it is extremely unlikely that the Pteridophytes have been derived from a Bryophytic ancestor with a sporophyte showing anything approaching the specialization of the moss-sporogonium, in which conducting tissues also occur. But it must not for this reason be supposed that the Bryophytes are of no interest in considering the problem of the evolution of the vascular system in Pteridophytes. We see among the former group, plants in the very act, so to speak, of developing a conducting system in response to vital needs, and others in the most various stages of its evolution in complexity. The conditions under which this evolutionary development occurred must have been practically identical with those to which the primitive Pteridophytic sporophyte was subjected—gradually.

increasing adaptation of a simple leafy form to terrestrial life. And the final result, as seen in the highest Polytrichaceae, is so strikingly like, even in many details, the state of things obtaining in the true vascular plant, as to furnish probably one of the completest and most interesting cases of homoplastic development in the plant-kingdom. It can hardly therefore be denied that the study of the conducting system in Mosses is calculated to throw most valuable sidelights on the question of the evolution of the vascular systems of the higher plants.

Vaizey¹ has already investigated the conducting tissues of the sporogonium of Mosses with the object of throwing light on the evolution of these tissues among the vascular plants, but he was under the influence, as it seems to us, of curiously narrow and misleading morphological doctrine. He sets on one side the tissues of the gametophyte of the Bryophyta, since according to the antithetic theory of alternation of generation the plant-body in which they are developed is not homologous with the plant of the Vasculares, and goes on to claim that 'every one will admit that the tissues of the sporophyte of the Muscineae are homologous with those of the sporophyte of the Vasculares' (p. 275). He thus implicitly assumes not only that the sporophyte of the Pteridophytes has been evolved from a Bryophytic sporogonium, but that its differentiated tissue-systems have been evolved from the differentiated tissue-systems of the highly specialized moss-sporogonium, an assumption so extremely improbable that it may be dismissed without discussion. In our opinion, while there can be no question of homology in either case, the conducting tissues of the Bryophytic gametophyte are at least as instructive as those of the sporogonium from this point of view of comparison, first because foliage-leaf structures are involved, and secondly because in the Polytrichaceae at least, there is a rhizome which is physiologically and histologically a root, directly continuous with the aerial

¹ Journ. Linn. Soc. Bot., vol. xxiv, 1887-8.

stem and thus comparable with the primary root in Pteridophytes.

It is to Haberlandt's admirable work on the Anatomy and Physiology of the Mosses that we owe the great bulk of our knowledge on this subject. He not only discovered most of the important facts but gave them a clear interpretation in the light of evolutionary theory. The following notes are intended to supplement his work, both in the further elucidation of points in the histology of Bryophytic conducting systems, and in the discussion of the various evolutionary problems involved.

I. THE CONDUCTING STRANDS OF LIVERWORTS.

The great majority of Liverworts, as is well known, have no differentiated water-conducting tissues. Living, as many of them do, in constantly damp situations, or flourishing only during the season when they are surrounded by air with a very high relative humidity, and frequently covered with rain or dew, they are used to absorbing water over their whole surface. A certain number no doubt depend for their water supply, at least at certain periods, on what is absorbed through the rhizoids which fix the thallus to the substratum, but the whole of the under-surface is commonly provided with these rhizoids, and the entire body is seldom too large to admit of the ready conduction of the water so absorbed through the ordinary parenchyma-cells to every part of the plant.

In a certain number of cases, however, among the thalloid forms, either assimilating parts of the thallus (*Pallavicinia*, *Symphyogyna*, *Hymenophyton*), or specialized reproductive branches (Marchantiaceae), are raised considerably above the substratum, and then special means are often adopted to facilitate the conduction of water to these remote parts. It is not our intention to review the various adaptations in question, but rather to confine ourselves to the consideration of one of them—the differentiation of a definite conducting strand.

The occurrence of a strand of elongated lignified thick-walled cells in the midst of a Liverwort-thallus was first discovered by Sir William Hooker (British Jungermanniæ 1816) in *Jungermannia* (now *Pallavicinia*) *Lyellii*. Gottsche¹, in 1864, describes a similar strand in *Symphyogyna sinuata*, N. et M., and states that there are often two such nerves at the base of the frond, which run parallel for some distance and often diverge at the first bifurcation of the thallus. He found that the strands had no connexion with the 'receptacles' on which the sexual organs were seated. Leitgeb in Heft 3 of his classical 'Untersuchungen über die Lebermoose' describes the cells of this strand in *Symphyogyna*, and states that *Blyttia* (*Pallavicinia*) and *Umbra-culum* (*Hymenophyton*) have similar strands. He mentions the interesting fact that in the common formation of adventitious branches from the ventral side of the frond in these genera, the strand of the branch is discontinuous with the strand of the parent frond. He describes the cells of the strand as being elongated with pointed ends and fairly thick walls, provided with close-set 'pores,' which are arranged in spiral lines round the walls, so as to give the impression of spiral thickening on casual examination of a longitudinal section. Haberlandt² refers to Leitgeb's descriptions, and expresses the opinion that the strand in question may very probably possess both a mechanical and a water-conducting function. Spruce³ describes and figures a new species of *Symphyogyna* from Dominica, which he names *S. trivittata* on account of the three strands running parallel in the midrib of the thallus. Sometimes there are only two in the upper parts, owing to the running off without branching of one of the three at a bifurcation, in the same way as in *S. sinuata*.

Farmer⁴, in a study of *Pallavicinia decipiens*, Mitten, describes the strand of the rhizome of this plant as consisting of

¹ Triana and Planchon's Prodrômus Floræ Novo-Granatensis. Ann. Sci. Nat., 5^e sér., tome i, p. 182.

² Beiträge zur Anatomie und Physiologie der Laubmoose, 1886, p. 378, note.

³ Hepaticæ Elliotianæ, Journ. Linn. Soc., vol. xxx, 1893-5.

⁴ Studies in Hepaticæ. Annals of Botany, viii, 1894.

‘fibrous cells whose walls become much thickened, and exhibit a number of shallow pits on their walls, at first view resembling ordinary striation.’ He also calls attention to the discontinuity of the axile strands of the branches with that of the mother-axis, and explains it developmentally by reference to the fact that the apical cell of a branch, after cutting off a few segments which form short broad cells, remains dormant for some time before continuing its development. It is only after the resumption of growth that the inner cells of its segments undergo the frequent longitudinal divisions which result in the formation of the cells of the axile strand. Hence the latter is always separated from that of the mother-axis by the first-formed broad cells.

In the hope of obtaining some information as to the conditions of the evolution of primitive vascular systems, we have investigated representatives of all three of the genera in question (the only Liverworts in which these strands have as yet been found), and though our observations are not so complete as we could desire, partly owing to the difficulty of getting sufficient and satisfactory material, but even more because of the impossibility of making a study of these plants in their homes—they are very scattered and nearly all tropical forms—yet the results seem worth putting together in this place.

Of the three genera *Pallavicinia*, Steph., *Symphyogyna*, Nees et Mont., and *Hymenophyton*, Steph., the two first are placed by Schiffner¹ in the Leptothecaceae, the last in the Metzgerioideae, neighbouring families of Jungermanniaceae and Anakrogyneae. They differ in well-marked characters connected with the position and investment of the sporogonium, and it is perhaps most probable that the striking character they have in common—the possession of an axial strand—has developed independently in each genus. Each of the three genera, too, may be divided into two sections, of which one, presumably the more primitive, contains species with an undifferentiated thin flat and creeping thallus, while in the other the thallus

¹ Engler and Prantl's Pflanzenfamilien, i, 3 (Hepaticae), 1893.

has a creeping rhizome-like isodiametric basal part, and from it arise the flat assimilating fronds, each of which is often spread out by repeated shortened dichotomy into the shape of a fan. Farmer (op. cit.) has pointed out the probability of the parallel development of this habit in the different genera.

Of the less differentiated species of *Pallavicinia*, Schiffner separates two, *P. Blyttii* and *P. hibernica*, from the others, by reason of their having no axial strand in the midrib (subgenus *Mörckia*, Gottsche gen.). Of these the latter, as originally described by Hooker (op. cit., Plate LXXVIII), is mainly separable from *P. Lyellii*, the other British species, by this character, but there seem to be several distinct forms confused under the name *Hibernica*. In specimens from Hooker's type from Loughbray, Co. Wicklow, and in specimens from Yorkshire, of quite different habit, which Mr. W. H. Pearson tells us agree with Gottsche's *Mörckia hibernica* we have found the tissue of the midrib quite homogeneous without any trace of an axial strand. In another plant, however, kindly sent us by Mr. David McArdle of the Royal Glasnevin Botanic Gardens, from sandy flats at Malahide, Co. Dublin, under the same name, but which he places under var. β , *Wilsoniana*, Carrington, we find two distinct lateral strands in the midrib, each consisting, in transverse section, of about twenty cells which do not differ from the surrounding tissue in width or in the thickness of their walls. These walls, however, are distinctly brown in the unstained condition, and hold aniline stains more strongly than the surrounding tissue (Pl. I, Fig. 1). On longitudinal section many of the strand-cells do not differ greatly in shape from the neighbouring cells, but they tend to be longer, and some are of considerably greater length (Fig. 2). Occasionally a very thin colourless cross-wall may be seen, as at *a* in Fig. 2. In none of these cells have we found nuclei, though they often possess a little contents, apparently scanty remains of protoplasm, as far as can be made out from herbarium-material.

In the absence of living material on which to experiment, we regard these strands as very primitive water-conducting

channels. There can scarcely be a question here of mechanical function, and there seems to be no demand in this type of plant-body for a special channel for conducting formed food-substances, a process which is no doubt carried on efficiently enough by the cortical cells. On the other hand the position of these strands, and the fact that their walls though thin are lignified, agree perfectly with the strands found in the other members of the genus which do in fact conduct water. If the inference is sound, we have in this Malahide plant the most primitive water-conducting tissue or 'hydrom'¹ known. Whether Carrington's var. β , *Wilsoniana* is a good constant variety, and whether, if so, these strands are always found in it, are questions we are unable to answer. If the answer should be affirmative, the plant certainly deserves specific rank on the ground of this well-marked anatomical feature alone. If not, the conditions under which the strands appear would form the subject of an investigation of great interest.

As we could not hear of a certain locality for the cosmopolitan *Pallavicinia Lyellii* in this country, Professor Howe, of Columbia University, very kindly sent us living specimens from Van Cortlandt Park, New York. The thallus of this species is band-shaped and wavy, about 3 to 5 mm. or more broad, with a thick, cushion-like midrib, .5 to 1 mm. in diameter. In transverse section the midrib in the specimen examined, .5 mm. broad and .25 mm. deep, is seen to be nearly flat on the dorsal surface, which is continued into the wings; these consist of a single layer of nearly cubical cells packed with chlorophyll, and forming the chief assimilating tissue. On the ventral surface the midrib is rounded, and some of its surface-cells are extended into thin-walled rhizoids, though these are not very numerous, so that in many transverse sections none are seen. The cells of the surface-layer are small, square in section, and full of chlorophyll; the deeper cells are variable in size and shape, several of those nearer the surface being full of chlorophyll, but most having

¹ Potonié, Ueber d. Zusammensetzung der Leitbündel bei den Gefässkryptogamen. Jahrb. d. kön. bot. Gartens, Berlin, Bd. ii, 1883.

little or none. The midrib, in the not very strong specimens examined, was ten to twelve cells thick. Approximately in the centre of the rib, but rather nearer the dorsal surface, is a strand of narrow thick-walled cells, about twenty in the specimen here described (considerably more in the one represented in Fig. 3), the whole of the strand being about the width of a single large cell in the cortex of the rib. The thick walls of these cells are greyish in thin, and yellowish in fairly thick transverse sections. They appear perfectly homogeneous. In longitudinal section (Fig. 4) they are seen to be considerably elongated, and pointed at the ends, their walls being covered with small pits arranged on a spiral, making quite a small angle with the horizon. The pits are sometimes circular, but more often elongated, sometimes considerably, in the direction of the spiral. They are rather irregularly scattered over the walls, considerable areas being destitute of them. In sectional view they are seen to be perfectly normal simple pits. A $\frac{1}{12}$ inch oil immersion is needed for a clear view of their structure. Where a strand-cell abuts on a cortical cell the thinner wall of the cortical cell is seen to be quite without pits, but the adjacent wall of the strand-cell may be richly pitted (Fig. 4).

Here and there in the strand-cells, a little contents can be seen, which are no doubt the remains of protoplasm. All stages of the disorganization of this can be seen in longitudinal sections close to the apex. The strand-cells are formed, as might be expected, by longitudinal division of the inner cells cut off from the segment of the apical cell. They are differentiated very close to the apex.

A few experiments were made to find out whether the strand actually conducted water. A piece of the thallus was cut off, and its cut end dipped into watery eosin, to a depth of about 3 mm. After a certain time the lower part of the strand became visible through the cortical tissue of the midrib as a red thread. In one case it could be seen 1 cm. above the level of the solution in the course of 20 minutes, on a hot, sunny day; in another, on a grey, damper day, the same

distance was traversed by the solution in the course of half an hour, while in one hour the solution was visible in the strand, 14 mm. above the surface of the liquid. Similar results were obtained in other trials. A difficulty is introduced, however, by the running up of the eosin on the surface of the thallus, obscuring the view, and staining the tissue. The eosin also spreads very rapidly from the strand to the cortex of the midrib, so that the whole rib becomes coloured not far behind the highest level which the solution has reached in the strand. The above results are, however, sufficient to show that water certainly will rise in the strand more quickly than through the surrounding tissues, though very much more slowly than Haberlandt records for the conducting strands of the Mosses. We failed several times in getting the eosin solution to rise in the strands of newly formed upright branches which arise from the ventral surface of the midrib. This suggests that the discontinuity of their axial strands with that of the parent axis puts a real obstacle in the way of rapid conduction.

The strands of the other species of *Pallavicinia*, and those of *Symphyogyna* and *Hymenophyton*, agree with that of *P. Lyellii* in all essential respects. They may differ, though we have not examined enough material to be sure that such differences may not exist within a species, in the number and size of the cells, the thickness of the walls, and the number and inclination of the pits. These last characters, however, certainly vary in the same strand.

We had rather expected to find that the forms with a creeping thallus, and consequently with all their cells comparatively close to the absorbing rhizoids, would have less-developed strands than the forms with large assimilating axial fronds rising freely above a creeping rhizome. On the whole our results bear out this expectation. It is probable that more light would be obtained on such relations as these by a careful study of the plants under their natural conditions.

Of the sub-genus *Mittenia* (Gott.) Schiffner, in which there are stalked fronds arising from a rhizome-like base, we have examined *P. decipiens* from Ceylon, and have practically nothing

to add to Farmer's description. Some of the hydroids, as seen under a $\frac{1}{12}$ inch oil immersion, are shown in Fig. 4, and it will be clear that the pits are simple and quite normal, not specially 'shallow.'

Symphyogyna. Schiffner gives two sections, *Repentes* and *Erectae*, according to the difference of habit already mentioned. Of the former we have examined *S. sinuata* from Trinidad, and *S. Brasiliensis* from an elevation of 4,000 feet in Bolivia (Figs. 5 and 6). So far as can be made out from scanty herbarium-material the former is closely adherent to the soil along the whole length of its midrib, and has numerous root-hairs all over the ventral surface of the rib, while the latter has no root-hairs along its upper parts, which are quite free. Certainly *S. Brasiliensis* has a very much bulkier strand than *S. sinuata*, with wider thin-walled elements at the periphery, and narrow thick-walled cells in the centre. *S. trivittata* from Dominica has already been described and figured by Spruce (op. cit., p. 365, Plate XXX). The three strands, though not individually very bulky, must afford between them a considerable conducting channel. Here there is a certain differentiation into rhizome and frond, such as one often gets in *P. Lyellii* (cf. Farmer, op. cit., p. 35), and no rhizoids are to be found on the frond.

Of the section *Erectae* we have examined *S. podophylla* from the Devil's Peak, and also from Kooksbosch, S. Africa, *S. Hymenophyllum* from New Zealand, and *S. rhizobola* (Figs. 7 and 8), all with dichotomously branched fronds on long stalks. The frond-stalks of all three have stout strands, which may be circular or slightly flattened in the plane of the frond, and their elements are well provided with pits of the usual type. The rhizome of *S. rhizobola*, the ventral surface of which is covered with rhizoids (Fig. 8), has a distinctly weaker strand than the frond-stalk (Fig. 7).

Hymenophyton. *H. Phyllanthus* (New Zealand) has the habit of *Pallavicinia Lyellii*, and its somewhat flattened basal rhizome-like portion has a small compact strand (Fig. 9).

H. flabellatum (New Zealand) has a very long stalk to its

fan-shaped frond, and this has a bulky band-shaped strand (Fig. 10), while that of the rhizome is much smaller. As in *Pallavicinia decipiens*, and apparently all such cases, the stalk of the frond is a direct continuation of the rhizome-axis, the rhizome itself being a sympodium, and the strand of each new member of this being discontinuous with that of the last. The base of the new strand is particularly weak, and it becomes gradually stronger as it passes up to the base of its frond-stalk.

We think it will be admitted that the facts support in a general way the theory of the water-conducting function of these strands, a bulkier strand being found wherever the axis can no longer depend directly on absorption by rhizoids.

Before we leave the Liverworts, we may refer to the striking absence, so far as is known, of anything like a water-conducting system among the very numerous and successful leafy Jungermanniaceae, the external vegetative organs of which are often so highly developed. The general explanation probably lies in the fact that practically all these forms are accustomed to absorb water at all points of their surface, which, owing to the great division of the body into leaves and leaf-lobes, is very much greater, compared with the mass of the plant, than in the thalloid forms. We even find the same thing among many of the Mosses in which no water channels exist. As Haberlandt¹ has pointed out, such forms are either hydrophilous or xerophilous; in either case absorbing water over the whole surface when they do absorb it. It may be laid down as a general principle, in fact, that a specialized water-conducting channel is as a rule only developed where the region of absorption is localized.

There is, however, in at least one genus of the leafy Liverworts, and probably in others, an indication of the development of the other great form of conducting channel, which provides for the passage of formed food-substances. The case to which we allude is the strand of elongated cells forming a kind of 'midrib,' though only one cell thick, in the

¹ Beiträge, pp. 389-91.

leaf of *Diplophyllum albicans*. Our attention was called to this by Schiffner's Fig. 64 H, p. 112. The cells of the 'rib' are oblong, and at the upper end show a tendency to spread out fan-wise and come into contact with a number of the ordinary square cells of the leaf (Fig. 11). The elongated cells contain less chlorophyll than the square ones, and their end-walls, as well as here and there the side ones, are irregularly pitted. Below, the cells of the rib are continuous with the similarly elongated cells of the stem. The tissue of the stem is quite homogeneous, showing no trace of a special conducting strand.

The difference between such a case as this and the strands we have been hitherto considering will be sufficiently obvious. The strand in question is no doubt to be considered as rudimentary 'Leitparenchym,' the forerunner, as Haberlandt has shown, of the leptom of the higher forms. Instead of a localization of the regions of absorption we have here a localization of the regions of assimilation, and a special need for ready removal of the products of assimilation owing to the square shape and thick walls of the assimilative cells which form the bulk of the leaf.

II. THE HISTOLOGY OF THE RHIZOME AND AERIAL STEM IN *Polytrichum*.

Although W. P. Schimper¹, Fr. Unger², and P. G. Lorentz³ laid the foundations of our knowledge of the anatomical structure of the Mosses, Haberlandt, in his admirable work, *Beiträge zur Anatomie und Physiologie der Laubmoose* (Pringsh. Jahrb., 1886), was the first to give a satisfactory detailed account of the principal types of tissue-structure, and to explain their physiology, not only by acute reasoning from the anatomical data, but also to some extent by direct

¹ Recherches anatomiques et morphologiques sur les Mousses. Strassbourg, 1848.

² Beiträge zur Physiologie der Pflanzen, vii, 1801.

³ Grundlinien zu einer vergleichenden Anatomie der Laubmoose. Pringsh. Jahrb., vi, 1867.

experiment. Subsequent observers have added comparatively little to our knowledge of the histology of the stem of the moss-gametophyte. The most important papers are Bastit's *Recherches anatomiques et physiologiques sur la tige et la feuille des Mousses* (Rev. Gén. de Bot., tome iii, 1891), and a contribution by Coesfeld (Bot. Zeit., 1892) bearing exactly the same title as Haberlandt's classical work. The former contains descriptions of the anatomy of the aerial stem, foliage-leaves, rhizome, and scales of *Polytrichum juniperinum*, but while Bastit pays more attention than Haberlandt to the structure of the Polytrichaceous rhizome, and describes some important new features, he fails to connect his descriptions with Haberlandt's careful and thorough account of the minute histology of the aerial stem, with the result that our knowledge of this part of the subject is left in a vague and unsatisfactory state.

A reinvestigation of several species of *Polytrichum* with the object of getting a clear idea of the histology of the stem as a whole, has led to the discovery that the structure of the rhizome is considerably more complex than Bastit apparently suspected, the tissue-systems corresponding in many points with those that Haberlandt has described for the aerial stem.

Haberlandt and Bastit both described *Polytrichum juniperinum*, and the latter mentions that the other species are fundamentally similar. This is true in broad outline, but *P. commune* possesses distinctly better characterized and more highly differentiated tissue-systems than *P. juniperinum*, *P. formosum*, and *P. piliferum*, the other species we have investigated. For this reason it will be most convenient to describe pretty fully the features it presents, afterwards referring to the points of difference exhibited by the other species. Haberlandt (op. cit., pp. 366, 368) drew attention to the fact that in relation to the stereom, the rhizome of Polytrichaceae has a distinctly root-like structure. As a matter of fact, the resemblance to the root of a vascular plant extends in a most striking way to nearly all the tissue-systems.

Cortex. The rhizome, which may be roughly circular in

transverse section, but is more often triangular with rounded corners and slightly convex sides, is covered by a small-celled and rather thick-walled surface-layer, which bears the long, thick-walled, and very brittle rhizoids, often in such great numbers as to form a dense matted investment, as thick as half the diameter of the rhizome. The scales described by Bastit are attached to the projecting corners or ridges. We have not been able to make out the exact distribution of the scales, but they appear to be inserted at considerable intervals along the ridges. Their structure is well described by Bastit (op. cit., pp. 352-5, Figs. 44-6). We did not succeed, however, in the midrib of the scales borne by the rhizome proper, in distinguishing his 'bundle' from his 'hypodermic zone.' We find that the midrib consists entirely of elongated thick-walled cells, continuous with those of the hypodermal strand occupying the ridge to which the scale is attached (Figs. 13, 14). Of Bastit's 'cellules allongées en forme de tibia' (p. 354), evidently equivalent to Haberlandt's 'siebröhrenartigen Zellreihen,' we can find no trace in these scales. Possibly Bastit's description refers to scales higher up the stem, showing a transition to the structure of the foliage-leaf.

Below the superficial rhizoid-bearing layer are two or three layers of living cortical parenchyma, thin-walled and polygonal in transverse section. This tissue is interrupted opposite the three ridges by the sclerenchymatous cells of the three hypodermal strands. The extent to which these strands are developed varies very much in different rhizomes, and in different parts of the same rhizome. They are usually quite massive (as in Bastit's Fig. 41), and their cells have very thick and strongly lignified walls. In other cases their development is much feebler, the walls of their cells being only slightly thicker and darker than those of the ordinary cortical cells. The cells of the hypodermal strand are distinctly prosenchymatous, with pointed ends. They are living cells sometimes containing starch.

As we pass radially inwards from the thick-walled hypodermal strand, we come to cells of greater diameter, and with

thinner and less lignified walls. On transverse section these cells usually show no sharp line of separation from those of the hypodermal strand, and form together with it a wedge-shaped mass of tissue gradually narrowing inwards which we propose to call the *radial strand*. In radial section its inner cells are shorter than those of the hypodermal bundle, and have horizontal end-walls. They often contain starch.

At the inner limit of the cortex comes the layer of strikingly large, radially elongated cells (*end.*, Pl. I, Figs. 12, 15; Pl. II, Figs. 16–18) described by Haberlandt and Bastit. Their radial diameter is often nearly twice as great as the tangential. A striking point has been missed by previous observers in the thickening and suberization of their radial and horizontal walls. This layer in fact presents all the characters of a typical root-endodermis. The thickened walls show the distinctive reactions, giving good differential staining with the aniline dyes (e.g. eosin and iodine green, or haematoxylin and safranin), and staining strongly with alcoholic solution of chlorophyll and alkannin.

The endodermis consists of three arcs, each occupying a side of the triangular rhizome. The ends of each are curved slightly inwards, and abut on the inner, larger cells of the radial strand by which the adjacent arcs are separated.

Central Cylinder. This consists of an inner compact mass of tissue with three broad projecting regions, the outer boundaries of which are parallel with the three sides of the rhizome, and three narrower furrows opposite its angles. This central mass consists mainly of very thick-walled elongated living cells, often with oblique end-walls, and containing here and there a little starch. Scattered among these stereids are elements of about the same diameter, but so far as we have seen quite destitute of contents. These are often united, as it appears on transverse section, in bands of two or three, the cells of each band being separated by extremely delicate cellulose walls, which are really, as Haberlandt has shown, the very oblique end-walls of the cells. The walls abutting on the stereids are also thin and unlignified. For

these elements, which are exactly like and continuous with those of the central strand of the aërial stem, and which Haberlandt has, we think, conclusively shown to possess at least a chiefly water-conducting function in the aërial stem, we propose to adopt Potonié's term *hydroid*, the water-conducting tissue, as a whole, being called *hydrom*¹. The above facts relating to the central cylinder have all been described by Haberlandt in *P. juniperinum* (op. cit., pp. 369-70). Bastit fails to distinguish properly between the hydroids and stereids.

In most cases the three-lobed central strand is clothed with a layer of fairly thin-walled living cells. Round each broad projecting lobe this layer abuts immediately on the arc of endodermis, and may fairly be considered as of the nature of a pericycle². Its cells are considerably elongated, with

¹ Potonié, Ueber d. Zusammensetzung der Leitbündel bei d. Gefässkryptogamen. Jahrb. d. k. bot. Gartens zu Berlin, ii, 1883. As a term in physiological anatomy we think this is preferable to Haberlandt's word *hadrom*, which also includes associated parenchyma, since it directly expresses the function of the tissue in question rather than the non-essential character of *stoutness*. Oltmanns (Ueber die Wasserbewegung in der Moospflanze, Strasburg, 1884) objected to Haberlandt's interpretation of this tissue as mainly water-conducting, but on insufficient grounds, which Haberlandt has sufficiently refuted. Coesfeld (Beiträge zur Anatomie und Physiologie der Laubmoose, Bot. Zeit., 1892, p. 153) takes the same view, and states that the occurrence in these cells of oil and proteid, noted as exceptional by previous observers (Goebel, Die Muscineen, Schenk's Handbuch der Botanik, Bd. ii, p. 369, Breslau, 1887, and Haberlandt, op. cit.), is general, that small grains of starch also occur in considerable numbers, and that a thin lining of protoplasm can be demonstrated. He suggests that the cells have a water-storing rather than a water-conducting function. He produces however no valid criticism of Haberlandt's experiments. Our own observations, so far as they go, certainly do not indicate the general occurrence of organized contents in these cells, though we have often found in them a good deal of organic substance mainly of proteid nature, and in both *P. commune* and *P. juniperinum* we constantly found a number of cells with dense proteid contents in the upper part of the cylinder of the aërial stem close to the base of the sporogonium. We have not been able to plasmolyse these cells, but we have not investigated the question of the existence of a protoplasmic lining very carefully. It is possible that such may exist, but in any case no nucleus is present. We regard the importance of the water-conducting function of this tissue as established, but no doubt there is a good deal of work yet to be done on the physiological anatomy of these tissues.

² These pericyclic arcs are not continuous with the pericycle but with the hydrom-sheath of the aërial stem, owing to the way the transition takes place (see pp. 22 and 23). It should be noted that the layer we have called pericycle in the aërial stem is not, like these layers, stelar conjunctive, but is differentiated from the inner cortex.

quite thin horizontal or slightly inclined end-walls, and seem always quite destitute of starch. This pericyclic layer may be as much as two or three cells thick, and there may be no very sharp line of separation between it and the stereids of the central strand. It is often interrupted in places, so that genuine stereids come to abut directly on the endodermis. Thin-walled hydroids also are frequently mixed with the pericyclic cells, and may likewise abut upon the endodermis (cf. Haberlandt, op. cit., Taf. XXI, Fig. 18).

Each pericyclic arc follows the broad lobe of the central mass round its angles, and is continuous with the tissue lining the furrows. Haberlandt says nothing about the strands of tissue occupying the three furrows. Bastit calls them 'secteurs péryclicques,' and remarks that they scarcely differ from the tissue immediately external to them (i.e. the inner part of what we have called the radial strands). He also says, however, that the tissue in the furrow 'presents the same characters as the elements of the pericyclic zone of the aerial stem.' Since by 'pericyclic zone of the aerial stem' Bastit refers to Haberlandt's leptom, this last statement, together with the general root-like organization of the rhizome, suggested to us that sieve-tube-like cells might possibly be found in the furrows. This is actually the case. The centre of the bay is occupied by a little group of from six to eight polygonal cells with light yellow walls (*lept.*, Figs. 12, 18, 19). The two innermost of these are commonly larger than the others, and are separated by a particularly thick radial wall (Fig. 17). On transverse section these cells often possess thick granular contents giving proteid reactions, and so far as we have observed never contain starch. In longitudinal section they often show the perfectly typical characters of Haberlandt's 'siebröhrenartigen Zellreihen' (Figs. 15, 16, 16a). It does not appear that Bastit recognized this fact, notwithstanding his above-quoted statement.

This little group of sieve-tube-like cells (*leptoids* as we may call them) is surrounded by starchy parenchyma, to which we may apply Troschel's term *amylom*. To the inside and

on the flanks is the layer of cells lining the furrow and continuous with the pericycle. This layer is one or two cells thick, and corresponds in character with the pericyclic arcs, except that it is uninterrupted by hydroids, and so far as we have seen always contains starch, which the pericyclic arcs do not. To the outside of the bay the leptom is bounded by the inner narrower portion of the wedge-shaped radial strand, whose cells as already described are rather thin-walled and slightly lignified, somewhat broader than the leptoids and containing starch.

The most striking feature in the arrangement of the fairly complicated tissue-systems of the Polytrichaceous rhizome is undoubtedly its practically complete identity with that obtaining in the root of a vascular plant. We have (1) a surface-layer bearing root-hairs, (2) a cortex bounded internally by a well-differentiated endodermis, (3) a pericycle, though incomplete and often interrupted, (4) a compact central cylinder in which most of the stereom is localized, and exhibiting (5) a definitely tri-radial arrangement of the leptom, the hydrom-elements being scattered among the stereids in the promontories between the leptom-furrows as well as in the centre of the cylinder. The existence of central tracheae mixed with mechanical elements is a fairly common feature in the roots of Angiosperms, especially among Monocotyledons.

The only points in which the tissue-arrangement differs from the root-type are the incompleteness of the pericycle and endodermis. The former is not only often interrupted by hydroids abutting on the endodermis, but, like the endodermis itself, is not continuous outside the leptom-strands. At these three points the periphery of the cylinder is, in fact, broken by the wedge-shaped masses of thick-walled cells which we have called the radial strands, leading out to the three hypodermal or scale-trace strands. In these last tissues we meet with the stem-nature of the rhizome, as shown by its bearing foliar organs.

The tissues of the central cylinder, i.e. everything within the endodermis and radial strands, may be classified into

(1) Hydrom, (2) Leptom, (3) Conjunctive tissue, which last we may again divide into (*a*) stereom (central), (*b*) unligified cells without starch (pericyclic arcs), and (*c*) unligified cells with starch (*amylom*, situated between hydrom and leptom). The fundamental resemblance of this (except for the curious fact of the absence of starch from the pericyclic arcs) to the arrangement of tissues in the stele of a vascular plant is sufficiently clear, though the differentiation is distinctly less, especially between the different regions of conjunctive. The entire absence of pits, so far as we have observed, from the tissues of the rhizome is a curious character. It is, however, to be noted that the end-walls of all the comparatively thick-walled cells, except the hypodermal fibres, are much thinner than the side-walls. This is especially the case in the regions of conjunctive *b* and *c*, and in the inner cells of the radial strand. It is to be presumed that the last two tissues carry on a large part of the carbohydrate conduction. The pericyclic arcs, we imagine, must be concerned in the conduction of nitrogenous compounds, for though its cells are not differentiated as leptoids, the only obvious difference is the absence of bulging at the junction of two cells, and the somewhat greater thickness of their end-walls.

The constancy of starch-distribution in all the sections we have examined, not only in the rhizome but in the aërial stem, is a striking character. Without this indeed, it would be impossible to separate the slightly differentiated tissues to the extent we have been able to do. We have examined plants in different conditions, both when growth was actively proceeding and when it was at a standstill, shoots in a purely vegetative state and others during the development of the sporogonium, but though there is great variation in the *amount* of starch present, we have found it constantly present in some tissues and constantly absent in others. We have never seen starch in leptoids although Haberlandt says it occurs in them when conduction is slow. We find that starch increases very much, cramming the characteristically

starchy tissues, in plants kept shut up in a tin box for a fortnight.

The rhizomes of the different species of *Polytrichum*, according to Bastit, are all built up on fundamentally the same type, and this certainly holds good of the species we have examined. *P. juniperinum* and *P. formosum* (Fig. 19) come very close to *P. commune*, but are smaller and with rather less well differentiated tissues. The hypodermal strands are certainly less massive and with thinner walls, while the cells of the inner part of the radial strands are frequently only to be distinguished from the adjacent cortex by their smaller diameter, especially in *P. juniperinum*. The furrows are on the whole shallower, and hydroids very frequently abut on the endodermis. The outlines of the hydroids are strikingly angular in transverse section, and the angles where the thin, very oblique end-walls are inserted are frequently re-entrant. In *P. juniperinum* the thickness and lignification of the walls of the stereids decrease gradually as the periphery is approached, while the diameter of the cells increases, so that the living cells bordering the endodermis are often quite wide. In *P. formosum* the diameter of the stereids is more constant throughout, but their lignification and the thickness of their walls fall off in the same way. In neither case is it easy to separate a distinct pericycle. Differentiation of the conjunctive has not progressed so far as in *P. commune*.

P. piliferum is much smaller and with much feebler tissue-differentiation. The hypodermal strands are little developed and in places not distinguishable on transverse section. The inner cells of the radial strand are scarcely distinguishable from the adjacent cortex. The furrows are much shallower, sometimes hardly distinguishable. The hydrom-stereom strand is small and often irregular in outline. The stereids are fairly thick-walled but very variable in diameter, the peripheral ones being quite wide. The hydroids are very few in number, of the same type as in *P. juniperinum* and *formosum*.

It is a striking fact that all these species, however badly differentiated in other respects, have the endodermis well marked.

The transition of the rhizome to the aerial stem takes place very slowly, and begins very low down, so that the typical anatomical characters of the rhizome are lost long before there is any alteration in external features. For this reason it is necessary to dig up the plants very carefully and obtain a considerable length of rhizome in order to get sections showing the typical features. A section of *P. juniperinum* cut three-quarters of an inch below the beginning of the strictly subterranean rhizoid-bearing portion will often still show transitional phenomena.

The anatomical features of the transition have in the main been correctly described by Bastit (op. cit., pp. 356–360, Figs. 47–50), though his curious terminology and apparent want of appreciation of the real nature of the tissues involved considerably detract from the value of his account.

The first thing that happens is the lateral extension of the hypodermal strands. These eventually meet and become continuous with the sclerenchymatous outer cortical zone of the aerial stem, as Haberlandt and Bastit have stated. This zone is already continuous, occupying the whole of the cortex down to the endodermis, before the stele loses its typical rhizome character. Meanwhile the hydroids have increased in size and number, and the larger ones tend to concentrate in the centre of the stele while the stereids correspondingly become fewer. At this stage the leptoid groups are extremely obvious, one or two of the elements often becoming very large, and their walls, which are pale straw-colour, standing out very clearly from the dark chestnut-brown walls of the surrounding tissue. The endodermis begins to lose its characters, and, together with the cells of the inner part of the radial strand, forms the foundation of the inner cortex of the aerial stem. The sides of the stem flatten so that it becomes a three-sided prism. The stereids of the stele decrease to very few so that the files of hydroids

come into lateral contact, separated by thick walls. The central hydrom-stereom mass is now surrounded by a complete investment, one or two cells thick, of starch-containing parenchyma with dark brown walls, continuous below with the pericycle and the starchy tissue internal to the leptom, and forming the characteristic starchy *hydrom-sheath* of the aërial stem. The furrows become shallower and gradually the hydrom-strand, which has by now lost the whole of its stereom, becomes cylindrical. At this level the files of large hydroids which occupy the centre of the stele are surrounded by scattered smaller ones, continuous with the small hydroids found in the pericycle of the rhizome, and these are mixed with moderately thick-walled starch-containing cells abutting on the dark brown hydrom-sheath. Higher up, these last cells are lost or absorbed in the hydrom-sheath, while the small hydroids increase in number and form the peripheral *thin-walled hydrom-mantle* of the stele of the aërial stem. The three leptom-strands are now completely outside the circumference of the cylindrical hydrom, from which they are separated by the fairly broad starchy sheath already described (Fig. 22). Externally they are bounded by smaller cells, which are often, however, not clearly distinguishable from those of the inner cortex. At this time each leptom-strand consists of two or three leptoids only, and it is only at a considerable higher level that the number is multiplied and lateral extension of each group begins, finally resulting in their fusion to form a complete peripheral leptom-mantle. Bastit figures this as taking place much lower down, before the peripheral ring of cortical stereom is complete (op. cit., Figs. 48, 49, p. 358), and he altogether misses the stage at which the still isodiametric leptom-strands are entirely outside the cylindrical hydrom.

Side by side with the lateral extension of the leptoids to form a complete leptom-mantle, the small peripheral hydroids increase in number and eventually form a complete wide zone round the larger central ones. At the same time obvious leaf-traces begin to appear in the cortex, in direct relation

to the appearance as we pass upwards of the two mantles of the stele. Thus we pass to the typical structure of the leafy aërial stem.

AËRIAL LEAFY STEM.

The histology of this has been described by Haberlandt and Bastit in *P. juniperinum*, and by Coesfeld in *P. commune*. To the former we owe the first good detailed account of its structure. Haberlandt finds the peripheral hydrom-mantle surrounded by a layer of leptom three or four cells thick, composed of (a) wider sieve-tube-like cells, and (b) narrower cells with oblique end-walls corresponding with 'cambiform' (elongated phloem-parenchyma) in Angiosperms. The only other differentiation in the leptom is the suberization of the walls of the inner layer or two of cells. These suberized cells do not, however, according to Haberlandt, differ in form or contents from the outer unsuberized layers (cf. his Fig. 11, Taf. XXIII). We find, on the contrary, in agreement with Strasburger¹, Goebel², and Coesfeld³, that the peripheral hydrom-mantle is immediately surrounded by one or two layers of cells with dark brown suberized walls and starchy contents, in fact an amylom-layer—the walls of which, according to Coesfeld (op. cit.), are folded like those of an endodermis—our *hydrom-sheath*, while immediately outside this comes a layer interrupted here and there by starchy cells, and rarely more than one cell thick, of typical sieve-tube-like cells or leptoids with no starch and light walls—the *leptom-mantle*. This is strangely neglected by the recent writers on the subject, Coesfeld (op. cit.) and Strasburger (Bot. Practicum, 2nd ed., 1897), although it is extremely easy to distinguish in *P. commune* on staining with iodine. External to the leptom-mantle comes a layer of cells shading into the inner cortex, but of rather less diameter, with rather thinner, nearly colourless walls, and particularly abundant starchy contents.

¹ Botanisches Practicum, 1st ed., 1884, p. 304.

² Muscineen, in Schenk's Handbuch, ii, p. 369.

³ Beiträge zur Anat. u. Phys. der Laubmoose, Bot. Zeit., 1892.

This layer, barely differentiated from the inner cortex, we propose to separate as a *rudimentary pericycle*. It is not, however, continuous with, and clearly has a different origin from what we have called the pericyclic arcs of the rhizome. It is considerably interrupted by the incoming leaf-traces.

Bastit calls the hydrom 'moelle,' the hydrom-sheath 'péricycle intérieur,' and the leptom (as limited by us) 'péricycle extérieur.' He does not recognize the existence of sieve-tube-like cells at all, but mentions the fact that the 'péricycle intérieur' has starchy contents. The three terminologies arranged in parallel columns may be of assistance to the reader.

<i>Haberlandt.</i>	<i>Bastit.</i>	
Hadrom	{ Moelle intérieure	Central thick-walled hydrom-cylinder.
	{ Moelle extérieure	Peripheral thin-walled hydrom-mantle.
Leptom	{ Péricycle intérieur	Hydrom-sheath.
	{ Péricycle extérieur	Leptom.
Rinde	Écorce	{ Rudimentary pericycle. Cortex.

Our Figs. 20 and 21 will enable the reader to distinguish the different tissue-systems.

The constitution of the stele cannot be properly understood without reference to the leaf-traces, to which we now turn.

The general features of the bundle of the leaf of *Polytrichaceae* have been well described by Haberlandt in *Atrichum undulatum* (pp. 402-4) and by Bastit in *P. juniperinum*. The former describes the living cells of the bundle as 'Leitparenchym,' while the latter describes them as 'cellules en forme de tibia.' We are able to confirm Bastit's observation. In a transverse section of the leaf of *P. commune*, the living cells of the bundle form three rows parallel to the surface of the leaf. The cells of the middle row have the largest transverse section, are lined with a thin layer of protoplasm and contain no starch, while the top and bottom rows consist of slightly smaller cells (amylom) containing starch. At the corners of the cells between the rows are the two series of

very narrow hydroids, square in section, each abutting on four or usually five living cells (cf. Bastit, Fig. 2, Pl. XIII).

In longitudinal section the middle row of living cells is clearly seen to consist of leptoids, many though not all of the cells having the characteristic bulging ends and very thin transverse walls (Bastit, Pl. XIII, Fig. 4 in *P. juniperinum*).

Though many of the facts relating to the passage of the leaf-traces through the cortex have been described by Haberlandt (pp. 404-5) and Bastit, we have thought it well to describe the histological features fully, as their accounts are by no means complete, and a full understanding of the relations are necessary to an appreciation of the morphological nature of the different tissues of the conducting system.

On entering the cortex the leaf-bundle at first maintains its general form, though the characteristic shape of the leptoids in longitudinal section does not appear to be maintained during their passage through the cortex, and the inner layer of accompanying starchy cells frequently becomes somewhat irregular.

The outer and inner rows of starchy parenchyma have dark brown walls, while the light yellow walls of the leptoids stand out clearly against the thick dark brown walls of the outer cortex.

No marked change of form is noticeable on the transverse section till the VIIth trace (Fig. 20) counting inwards is reached. This trace has arrived at the somewhat smaller-celled and thinner-walled abundantly starchy layer forming the extreme internal layer of the cortex, which we propose to call a rudimentary pericycle (see above, *rud. per.*, Fig. 20).

As the inner layer of living cells of the trace reaches this, its cells change their character, becoming larger and lighter walled, in fact assuming the characters of the pericycle itself. At the same time the whole trace lessens its tangential length, becoming isodiametric instead of band-shaped, increasing the number of its leptoids from a single band of about six to nine or ten in a group, while the hydroids begin to lose their regular arrangement (Fig. 20, VIII and IX). The trace now

pushes through the stelar leptom-mantle and its inner (pericyclic) cells just mentioned join the dark-walled starchy hydrom-sheath. At the same time the hydroids also rather suddenly increase in number, collecting together in a strand roughly circular in section (XI and XII), while the leptoids appear to go off right and left in two groups to form part of the stelar leptom-mantle (X and XI). The outer layer of dark brown-walled starchy parenchyma belonging to the trace is still seen immediately external to and on each side of the hydroid-strand (XII). The latter now pushes forwards and joins the peripheral thin-walled hydrom-mantle with which its elements are identical in every respect, the starchy sheath of the leaf-trace joining the starchy hydrom-sheath of the stele in the same way (traces XIII and XIV)¹.

The most important fact to notice in this series of phenomena is the formation, as it were, of the whole of the stem-stele with the exception of the central thick-walled hydrom-cylinder and the rudimentary pericycle, from the bases of leaf-traces. All the elements of the stelar leptom at a given level can be accounted for as belonging to about six leaf-traces (in Fig. 20 those numbered X to XV inclusive) completing in the phyllotactic spiral about two circuits of the stele. The same may be said of the elements of the hydrom-sheath and of the thin-walled peripheral hydrom-mantle, though here, owing to the complete merging of the incoming

¹ Coesfeld (op. cit., p. 164) states that the large cells of the trace (our leptoids) enter the peripheral ring of the hydrom-cylinder together with the hydroids. We do not believe that this is the case. The cylindrical strand of thin-walled elements surrounded by a starchy sheath and representing the base of a trace (XI and XII) certainly differs from the row of isolated hydroids of the band-shaped upper portion of the trace; the elements are much more numerous, are wider, and often have disorganized contents. But they always differ from the leptoids of the upper part of the trace and of the leptom-mantle in having no nucleus. Together with the cells of the peripheral thin-walled mantle of the central part of the stele, with which they are continuous and identical in character, they represent, according to our theory (see third section), the last part of the hydrom-system to be evolved, the final connexion between the detached leaf-trace and the hydrom of the primitive stele. Their differentiation appears to be more rudimentary than that of the other hydroids. It may be that they help to conduct formed material under some circumstances, but they clearly do not belong to the regular leptom-system.

hydroid strands in the stelar mantle, the elements actually belonging to the different traces can no longer be distinguished. We have in fact a stele entirely composed (with the above-noted exceptions) of leaf-traces, a state of things which corresponds to that obtaining when all the bundles in the stem of a Phanerogam are 'common bundles.' The implications of this will be discussed in the third section of these 'Notes.' Coesfeld (op. cit., pp. 156-8) objects to the separation of the central thick-walled strand from the outer thin-walled mantle. But the sharp line of separation between the two is obvious enough as an anatomical fact in *P. commune* and often in *P. juniperinum*, and in all the species a difference can be made out between the peripheral and the central tissue. It is perfectly true that 'the whole tissue of the central strand is homogeneously laid down behind the growing point,' but that is only what one always finds when two tissues of similar form are developed in contact. 'Developmental' facts of that kind can have no bearing on morphological conclusions. Variability in thickening of the walls of the peripheral zone, both in different species and also in different stems belonging to the same species, certainly exists, and sometimes the mantle shades gradually into the central strand, but this assimilation of the neighbouring cells of regions primitively distinct is again a familiar enough fact throughout plant-histology, and cannot we think be set against the kind of general evidence we have adduced in the third section of these Notes.

The aerial stems of *P. juniperinum*, *formosum* and *piliferum*, like their rhizomes, resemble that of *P. commune* in all fundamental respects, though their tissues are rather less well-differentiated, on the whole thinner-walled, and consequently more difficult to separate. The diameter of the hydroids in the central thick-walled cylinder of *P. juniperinum* is less than in *P. commune*. The peripheral hydroid mantle is thicker-walled, the distinction between the two being less sharp. The other layers of the stele are well marked, the pericycle being distinctly thinner-walled and more sharply

separated from the cortex than in the stouter species, while the cortex itself is distinctly thinner and the number of leaf-traces present at a given level decidedly less, only seven to ten being found outside the hydroid mantle (cf. Bastit, Fig. 30 p. 269). There is but a single row of hydroids in the leaf-bundle between the leptoids and the lower (outer) row of starchy 'Leitparenchym,' which last when it enters the cortex has, as Haberlandt states, much lighter walls than in *P. commune*. The inner row of 'Leitparenchym' loses itself in the cortex. The changes in form of the trace as it passes into the cylinder are quite similar to those in *P. commune*.

P. formosum has very much thinner-walled tissues than either of the two preceding species. Its central thick-walled hydrom-strand has a considerably smaller diameter, but its elements are very like those of *P. commune*. The peripheral hydrom-mantle is very thin-walled and relatively very broad, and the hydrom-sheath is well differentiated. The tissues outside this, however, including all but the surface-layer of the cortex, are quite thin-walled, and the leaf-traces are not at first sight easy to distinguish. Their behaviour seems, however, to agree in essentials with that obtaining in the other species. There are not more than ten present outside the hydrom in any given section.

In *P. piliferum* the central cylindrical strand is large relatively to the diameter of the peripheral mantle, which is not very well differentiated from it. The hydroids are rather narrow. The hydroid-sheath is large-celled and thick-walled—very well-marked. The leptom-mantle is difficult to distinguish in the transverse section. The leptoids are comparatively few in number, their walls do not differ from those of the adjacent tissues, and their cells often contain granules which before treatment with iodine much resemble the starch of the other tissues. The leptoids of the mantle are seen, however, on longitudinal section to possess typical characters, and we have not found starch in them. On the other hand, the corresponding cells of the leaf-bundle and trace often do contain starch and show no differentiation from ordinary

'Leitparenchym.' The cortex is narrow and there are only about six or seven traces present outside the hydroid cylinder. These, except for the fact above noted, are of quite the same type as in the other species.

III. THE NATURE AND ORIGIN OF THE CONDUCTING TISSUES IN MOSSES.

Unger¹ was the first to compare the 'central strand' found in the great majority of moss-stems with the vascular strand of a higher plant, while he and Lorentz² discovered and described the leaf-bundle and the leaf-trace which sometimes continues the leaf-bundle downwards into the stem. Lorentz also discovered the remarkable fact that in some cases the leaf-traces end blindly in the cortex of the stem ('falsche Blattspuren'), while in others they join on to the central cylinder, as in the Vascular Plants ('echte Blattspuren'). Haberlandt, in his 'Beiträge,' advanced the whole subject enormously by giving definiteness to the previously vague attribution of the function of 'conduction' to these tissues, showing by experiment³ that the central strand of most moss-stems is a water-conducting channel, a rudimentary 'Hadrom,' and that in Polytrichaceae there are in addition tissues which function as conductors of plastic food-material, both carbohydrate and nitrogenous. In the second section of the present 'Notes' we have shown that in both rhizome and aerial stem there is a layer of starchy parenchyma between hydrom and leptom, just as in all vascular plants (Russow's 'Xylem-scheide' in Ferns), and also indications of a rudimentary pericycle outside the leptom of the aerial stem. In fact there are parallels among the Polytrichaceae for all the main categories of the stelar tissue of the true vascular plants with the exception of primary phloem-fibres, which are always rare.

¹ Beiträge zur Physiologie der Pflanzen: vii. Ueber den anatomischen Bau des Moosstammes. Sitzungsber. d. Wien. Akad., 1861.

² Grundlinien.

³ Ber. d. Deutsch. bot. Ges., 1883.

With regard to the evolution of this complicated system of conducting tissues, we can follow in the completest way the different stages of its differentiation.

The very earliest stages are not yet known among the Mosses themselves, but we find one type in the leaves of the leafy Liverwort *Diplophyllum*, and the other in the midrib of the thallus of the plant from Malahide at present known as *Pallavicinia hibernica*, var. β , *Wilsoniana*.

The first demand in a leafy plant vegetating in a damp atmosphere, and capable of absorbing water more or less over its whole surface, is probably for easy conduction of formed food-substances away from the leaves, rather than for the rapid supply of water to the leaves. The former is probably the function of the living elongated cells forming the midrib of the *Diplophyllum* leaf. The strands of prosenchymatous lignified cells in the midribs of the thallus in *Pallavicinia*, *Symphyogyna*, and *Hymenophyton* on the other hand are no doubt water-conducting, as we have shown in the case of *P. Lyellii*. Of these, the strands of *P. hibernica* β , *Wilsoniana* are certainly the least differentiated, and in all probability the most primitive. The cells of the strand are more elongated than the cortical cells, but their walls, though slightly lignified, are scarcely thicker than those of the cortex. They are not entirely destitute of contents, though we have not been able to make out that any of them possess a nucleus. Taking all the facts into consideration, in the absence of experiment it is probable that they function as a water-conducting channel. From this type we pass to the very long and narrow, thick-walled, lignified, and richly pitted elements typical of the central strand in the three genera named. These may be regarded as comparatively highly differentiated hydroids.

In the majority of the Mosses we have a similar stage of development in the central strand of the stem, except that here the hydroids are thin-walled and nearly always without pits. The leaves possess in their midribs elongated living cells, no doubt for the conduction of formed organic sub-

stances into the stem, and frequently hydroids associated with these. The leaf-bundles are not usually continued into the stem, the conduction in the stem itself of the formed substances coming from the leaves being carried on no doubt by the longitudinally elongated cortical cells. With regard to the conduction of water, it seems to take place, in some cases at least, across the cortex from the central strand to the bases of the leaves. But no doubt nearly all Mosses take up water through their leaves on occasion, while the aquatic, semiaquatic, and xerophilous types, as Haberlandt has shown, habitually do so, and possess no conducting system at all or one which is very feebly developed. It is probably owing to the retention of this primitive habit in many terrestrial Mosses that a more efficient water-conducting system is dispensed with. A certain number of terrestrial forms, however, have evidently felt the need of such a system. In *Mnium*, *Bryum*, and some species of *Splachnum*, the hydroids of the leaf-bundles are prolonged into the stem, but curiously enough, instead of crossing the cortex to join the central cylinder, they bend down and pursue a vertical course near the edge of the cortex, parallel with the long axis of the stem. These leaf-traces or their lower ends have their surface increased in various ways, i.e. they may be band-shaped or star-shaped in cross-section so as to present a larger surface to the cortical cells through which their water must come. In *Funaria hygrometrica* the leaf-traces take an obliquely radial course through the cortex, and approach the central cylinder, but usually just stop short of it, though in some cases they actually join it. Finally, in some species of *Splachnum*, in *Voitia nivalis*, and throughout the Polytrichaceae, the traces regularly join the cylinder.

The first thing that strikes one in considering this series of facts is the entire *separateness* in evolution between the primitive moss-stele and the leaf-bundles. Not only do they appear quite independently in the lowest types, but in the case of the (no doubt phylogenetically subsequent) continuation of the leaf-bundle back into the stem as a leaf-trace, the latter

is often quite unconnected spatially, though it clearly has a physiological connexion with the stele. It is only as a second thought, so to speak, that the establishment of a direct spatial connexion with the hydrom of the cylinder occurs to the trace, as being the simplest and most effective means of establishing a water-channel between the absorbing part of the stem and the leaf. In none of the higher plants, so far as we know, is anything similar found. However simple or complex the stele may be, the leaf-traces are always in direct connexion with it from the outset. This being so, it is difficult to believe that the stem-stele and leaf-bundle of the Mosses were evolved as parts of a single conducting system designed for a single purpose. The explanation that suggests itself to us is that the stem-stele originated in the first instance in order to supply the growing apex (cf. Strasburger, *Practicum*, xxi Pensum), the sexual organs, and particularly the developing sporogonium, with water; while the leaf-bundles originated quite independently, at first probably in the form of a median strand of 'Leitparenchym' (as in the Liverwort *Diplophyllum* and many Mosses), to conduct the products of assimilation away from the leaf, hydroids being afterwards associated to facilitate the passage of water to, and possibly from, the leaf, as alternately the transpiring and absorbing organ. In those forms which adopted a purely terrestrial habit, rooted in soil and expanding their leafy shoots in air which was often relatively dry, the current of water up the stem became more constant and the leaves became more regularly and to a greater extent transpiring organs. A demand for easier conduction from stem to leaf was felt and was met by the continuation of the water-conducting part of the leaf-bundle into the stem, where it was expanded in various ways (*Mnium*, *Bryum*, *Splachnum*) in order to present a larger surface to the cortical tissue, so that the leaf-bundle could indirectly tap the water in the central strand to greater advantage.

The habitats and habits of the plants belonging to these genera completely correspond with the existence of such

a demand, and Haberlandt has shown that conduction of water up the central strand and its passage thence into the leaf-traces and leaf-bundles, to meet the losses caused by transpiration, is a regular occurrence in *Mnium undulatum*. Finally, the more direct and efficient method of the establishment of continuity between the hydrom of the stem-cylinder and the hydrom of the leaf-bundle has been adopted in the case of other species of *Splachnum*, and in all the Polytrichaceae. In the last-named family we have, for the first time, the 'Leitparenchym' (some of the cells of which have now assumed the characters of a distinct leptom) also continued down, side by side with the hydrom, from the leaf-bundle into the leaf-trace, and so into the leptom-mantle and starchy hydrom-sheath of the stem-cylinder.

We think that the present theory is distinctly borne out in rather a striking way by the facts with regard to the constitution of the Polytrichaceous stem-cylinder set out at the end of the last section. The central thick-walled hydrom-cylinder is entirely independent of the leaf-traces and passes up to the top of the stem, where in the case of a sporogonium-bearing shoot it changes its characters, becoming thin-walled and often mixed with cells having dense proteid contents and envelopes the base of the sporogonium. This, in our view, is the primitive hydrom-cylinder of the stem of the gametophyte. The thin-walled peripheral hydrom-mantle, on the other hand, composed, as Haberlandt has pointed out, of elements identical with the hydroids of the leaf-traces, is as a matter of fact entirely formed from the bases of the hydrom-strands of these traces, and decreases in thickness upwards, ceasing to exist above the level of exit of the last of the traces; while below it rapidly diminishes after the lowest of the traces has joined the stele, and is represented in the rhizome by a very few elements in the region of the 'pericycle.' The peripheral hydrom-mantle is then a new formation, only arising with the attachment of the bases of the leaf-traces to the stem-cylinder.

Nearly the same may be said of the leptom-mantle, which

in the leafy stem is clearly enough composed of the bases of the leptom-strands of the leaf-traces. Traced upwards, however, after the passing out of the last of the leaf-traces, it is continued upwards together with the altered cells of the hydrom-sheath, which no longer contain starch: consisting of a layer of thin-walled elongated cells, often with dense proteid contents, but in which no typical leptoids can be detected, it overlaps the central hydrom and comes into contact at a higher level with the base of the sporogonium, to which no doubt it supplies plastic substances for the formation of the young spore-capsule. Below the lowest leaf-trace the leptoids, as we have seen, decrease in number very considerably, and the remaining ones are concentrated in three strands which run down into the furrows of the hydrom-stereom cylinder of the rhizome.

According to this view, the highly developed Polytrichaceous stele is in the aërial stem essentially double in nature and phylogenetic origin, consisting (1) of a central primitive hydrom-cylinder, originally developed and still serving to supply the apical bud, sexual organs, and sporogonium with water, and (2) of a double peripheral mantle of hydrom and leptom separated by a starchy hydrom-sheath (amylom), all three layers composed of the joined bases of leaf-traces and designed between them to conduct water to, and formed material from, the leaves.

The bearing of these considerations on the problem of the nature and origin of the primitive stele among the Pteridophytes, as we find it for instance among the Sphenophyllineae and Lycopodineae, is a very interesting question which we cannot here discuss at length. We can only suggest the possibility of two alternative explanations of such a stele. Assuming first for simplicity's sake the truth of the Bowerian theory, we may on the one hand suppose the primitive Pteridophyte descended from a form bearing a terminal fruit-body, rather of the nature of the sporogonium of an *Anthoceros*, and with a primitive hydrom-stele comparable with that of the Mosses, but supplying this fruit-body directly (since it

is developed in the sporophyte) instead of merely leading up to the base of the sporogonium. The lineal descendant of such a primitive hydrom-stele would then perhaps be seen in the central metaxylem of, for instance, *Sphenophyllum*, *Cheirostrobilus*, the *Lepidodendra* with solid steles, the monostelic *Selaginellas*, and (modified in various ways) in *Psilotum*, *Lycopodium*, &c. Added to this would be the bases of the leaf-traces, represented by the peripheral protoxylem-strands (separate, or as in *Lepidodendron* laterally confluent) and only evolved after the primitive sporophyte had thrown out leaves requiring a vascular supply connected with the main channel of the stem. The fact that they appear *before* the central xylem in the development of the individual stem would be merely in relation to the need for the early establishment of conducting channels to the leaves, a need which is universal in leafy vascular plants.

On the other hand we might suppose that the formation of leaf-structures requiring a vascular supply preceded the formation of a regular stele supplying the fruit-body, in which case the leaf-traces, represented in the first place by the protoxylem-strands, would be phylogenetically prior, and the central metaxylem would be a later formation, developed in the larger forms to furnish additional conducting channels to supplement the protoxylems in supplying the needs of the higher foliage-leaves and the sporophylls.

And if, setting aside the antithetic theory, we imagine the evolution of the sporophyte from the alternate generation of a homogenetic thalloid form, the elements of the problem are not essentially changed. It is still, as it appears to us, a question of the priority in evolution of leaf-structures on the one hand, or of a differentiated conducting strand on the other. In the latter case the metaxylem of the protostelic Pteridophyte may represent a primitive water-conducting strand in the centre of a thallus, such as we find in *Pallavicinia*; in the former, merely a later-formed supplement to the connected system of leaf-traces.

EXPLANATION OF FIGURES IN PLATES I AND II.

Illustrating Mr. Tansley's and Miss Chick's paper on the Conducting
Tissue-System in Bryophyta.

PLATE I.

Section I. The Conducting Strands of Liverworts.

Fig. 1. Transverse section of midrib of thallus of *Pallavicinia hibernica* (Hooker), S. F. Gray, *β. Wilsoniana*, Carrington, from Malahide, co. Dublin, showing two lateral strands of thin-walled but lignified, probably water-conducting cells. $\times 48$.

Fig. 2. Part of longitudinal section of the same, passing through one of the strands; showing elongated, slightly prosenchymatous shape, and scanty disorganized contents of the strand-cells. At *a*, very thin cross-wall in a long strand-cell. The lignification extends to parts of the walls of the neighbouring cortical cells. $\times 50$.

Fig. 3. Transverse section of midrib of thallus of *Pallavicinia Lyellii* (Hooker), S. F. Gray, showing central strand of narrow thick-walled hydroids, and a few rhizoids on ventral surface. $\times 110$.

Fig. 4. Parts of hydroids of *Pallavicinia decipiens*, Mitten, showing the often irregularly scattered pits. These are oval or elongated in surface view, with their long axes perpendicular to the long axis of the cell, or more often slightly inclined, so that the pits are arranged on imaginary spirals running round the cell. In section they are seen to be normal deep simple pits. Where a hydroid abuts on a cortical cell (left-hand hydroid and left-hand wall of right-hand group) the thin wall of the latter is quite unpitted, while the wall of the hydroid may be richly pitted. $\frac{1}{2}$ " oil immersion. $\times 500$.

Fig. 5. Transverse section of midrib of thallus of *Symphyogyna sinuata* (Sw.), Mont. et N., showing rhizoids on ventral surface of thallus and weak central hydrom-strand. $\times 100$.

Fig. 6. Transverse section of midrib of thallus of *S. Brasiliensis* (N. ab. E.), Mont., showing absence of rhizoids and bulky strand with cells of larger diameter at the periphery. $\times 100$.

Fig. 7. Diagram of transverse section of frond-stalk of *S. rhizobola*, showing relation of diameter of bulky strand to that of whole stalk.

Fig. 8. Transverse section of rhizome of the same, showing rhizoids and relatively weaker strand. $\times 100$.

Fig. 9. Transverse section of comparatively weak rhizome-strand of *Hymenophyton Phyllanthus* (Hooker), Dum. $\times 145$.

Fig. 10. Transverse section of bulky band-shaped strand of frond-stalk of *H. flabellatum* (Hooker), Dum. $\times 100$.

Fig. 11. Upper part of midrib of leaf of *Diplophyllum albicans* (L.), Dum., in surface view, showing elongated cells of rib with sparsely and very irregularly pitted walls and much fewer chloroplasts than the square cells forming the rest of the leaf. $\times 200$.

Section II. The Histology of the Rhizome and Aërial Stem in *Polytrichum*.

Fig. 12. Part of transverse section of the rhizome of *P. commune*. Central three-lobed hydrom-stereom cylinder: *hyd.*, hydroids; *ster.*, stereids; *per.*, pericyclic arc; *end.*, endodermal arcs. In the furrows the groups of *lept.*, leptoids, and *amyl.*, starchy tissue. Outside these, *i. r. s.*, the inner larger cells of the radial strands; *hyp. st.*, the hypodermal strands; *cor.*, cortex; *rh.*, rhizoids. $\times 145$.

Fig. 13. Transverse section of hypodermal strand of same, immediately above point of insertion of a scale; midrib of latter is seen to consist of thick-walled tissue like that of hypodermal strand. $\times 145$.

Fig. 14. Radial longitudinal section of same, showing insertion of scale and hypodermal (scale-trace) strand giving off branch which becomes midrib of scale: *c.c.*, central cylinder.

Fig. 15. Part of radial longitudinal section of same, passing through lobe of cylinder; references as in Fig. 12. $\times 245$.

PLATE II.

Fig. 16. Part of longitudinal section of same, passing through edge of lobe and furrow of cylinder; references as in Fig. 12. $\times 145$.

Fig. 16a Part of three leptoids from Fig. 16. $\times 570$.

Fig. 17. Transverse section of a leptom-strand of rhizome of same: *lept.*, leptoids; *amyl.*, amyloim; *i. r. s.*, inner cells of radial strand. $\times 385$.

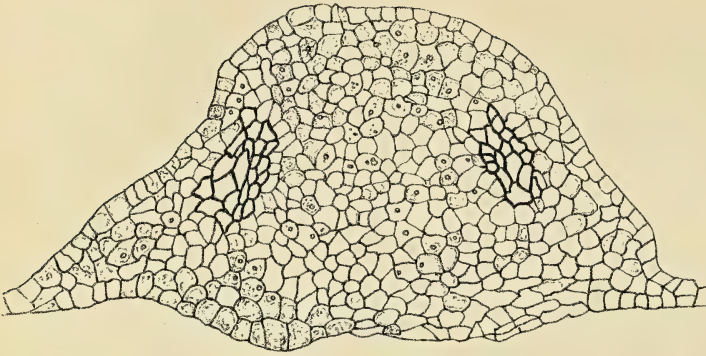
Fig. 18. Transverse section of central cylinder of another rhizome of same, showing gradual transition from stereom to pericycle. References as before. $\times 145$.

Fig. 19. Part of transverse section of rhizome of *P. formosum*, showing shallowness of furrows, absence of well-marked pericycle, angularity of hydroids, and hydroids abutting on endodermis; references as in Fig. 12.

Fig. 20. Transverse section of central tissues of aërial leafy stem of *P. commune*, showing entrance of leaf-traces into the mantles of the central cylinder: *cor.*, cortex; *rud. per.*, rudimentary pericycle; *hyd.*, hydroids of leaf-traces; *hyd. e.*, peripheral hydrom-mantle; *hyd. i.*, thick-walled central hydroids of cylinder; *lept.*, leptoids of leaf-traces and mantle; *amyl.* (amyloim), starchy conducting parenchyma of leaf-traces; *hyd. sh.*, hydrom-sheath. The leaf-traces are numbered from without inwards in Roman figures.

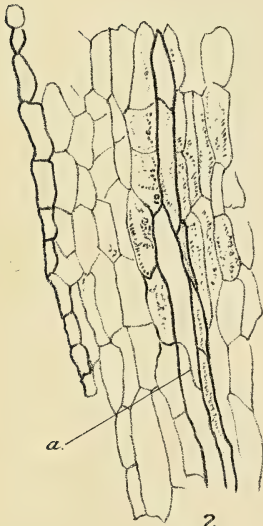
Fig. 21. Part of radial longitudinal section of leafy aërial stem of *P. commune*: *cor.*, cortex; *rud. per.*, rudimentary pericycle; *lept.*, typical leptoids; *hyd. sh.*, hydrom-sheath packed with starch; *hyd. e.*, peripheral thin-walled hydrom-mantle; *hyd. i.*, central thick-walled hydrom. $\times 200$.

Fig. 22. Transverse section of transitional region of *P. commune*: *lept.*, showing the three isodiametric leptom-groups; *hyd.*, hydrom-strand which has now lost all stereids; *hyd. sh.*, hydrom-sheath; *x.*, small cells with starch bounding leptom-groups. $\times 230$.



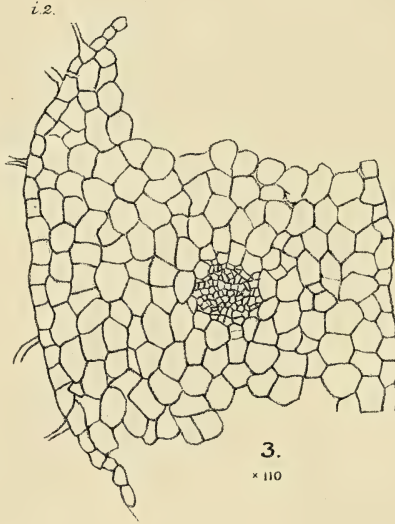
1.

x 48



2.

x 50



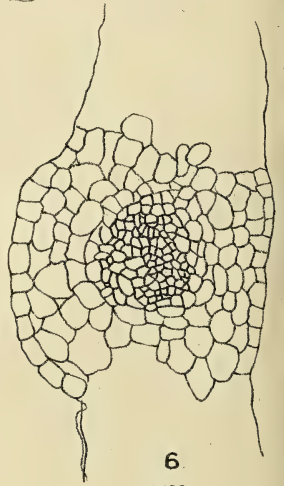
3.

x 110



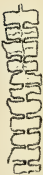
5.

x 100



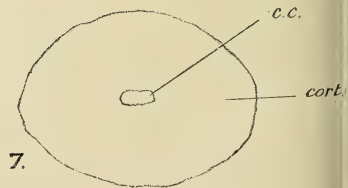
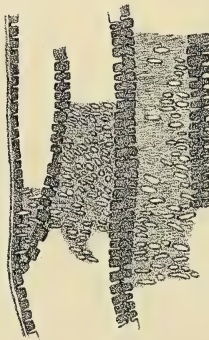
6.

x 100

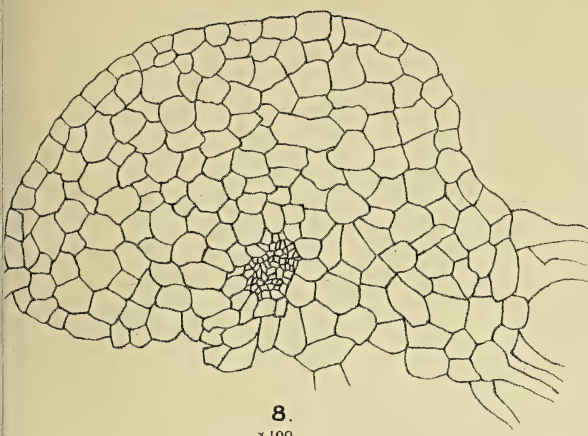


4.

x 500



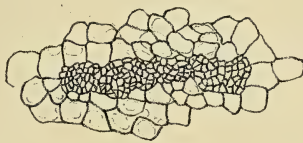
7.



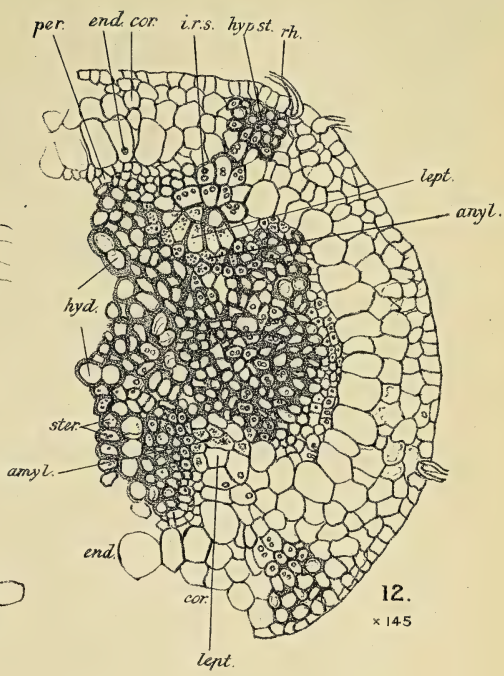
8.
x 100



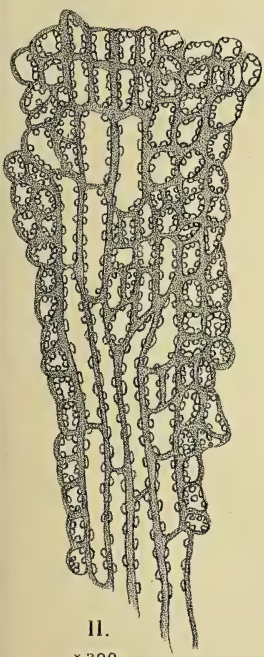
9.
x 145



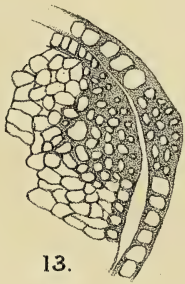
10.
x 100



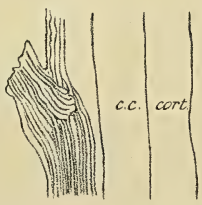
12.
x 145



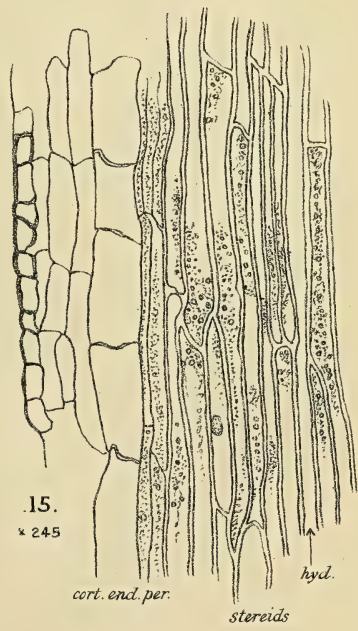
11.
x 200



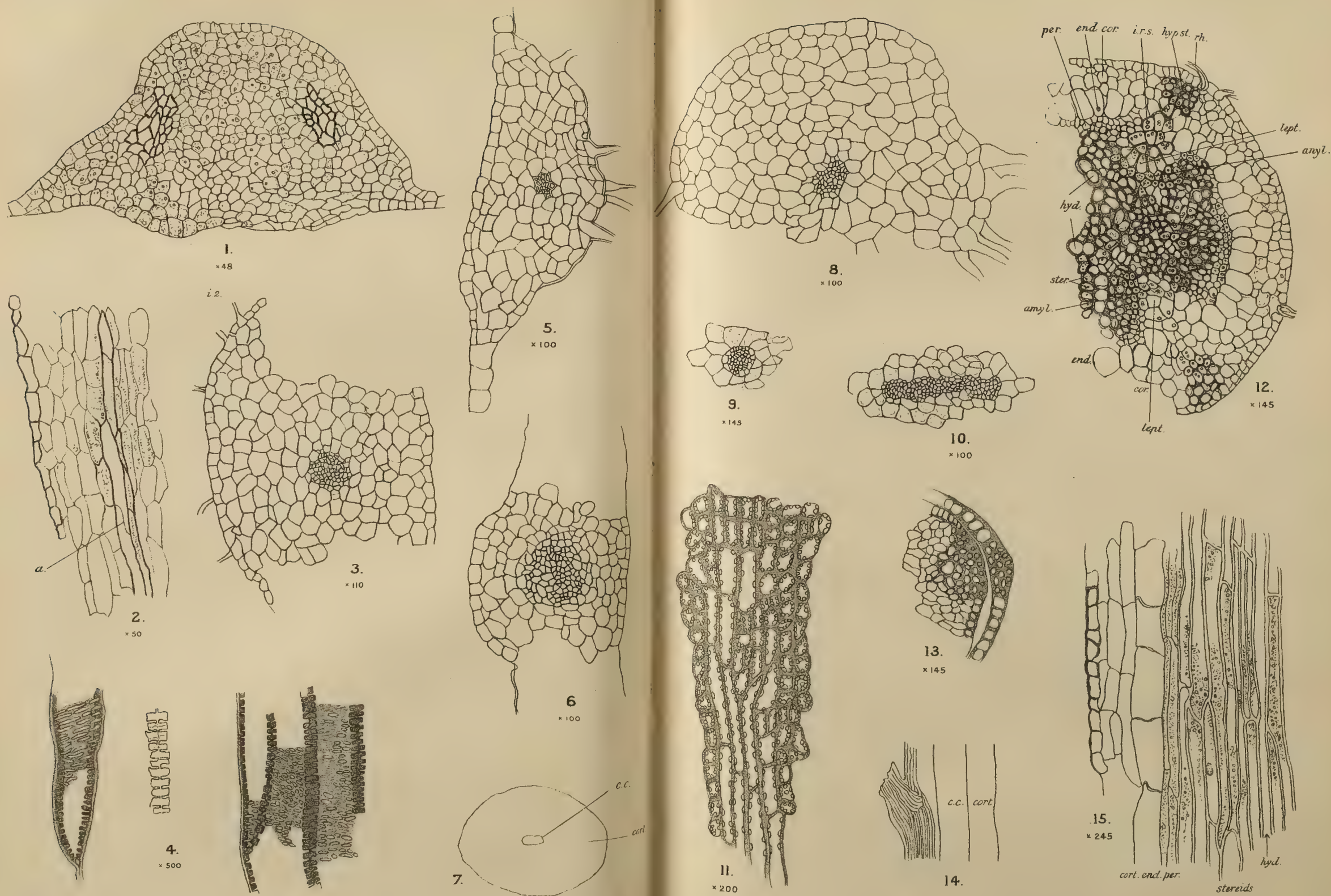
13.
x 145



14.



15.
x 245





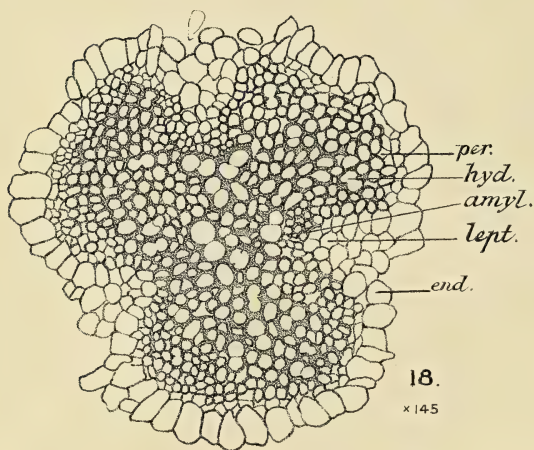
16.
 $\times 145$



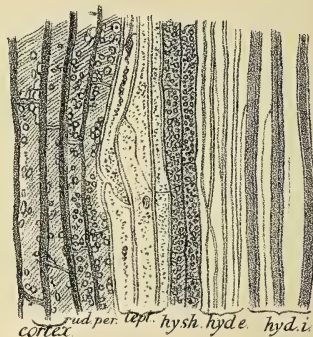
16a.
 $\times 570$



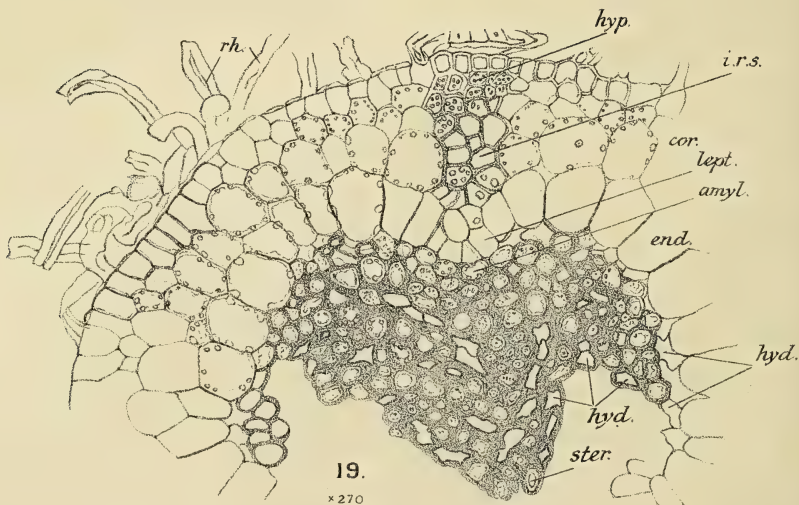
17.
 $\times 385$



18.
 $\times 145$

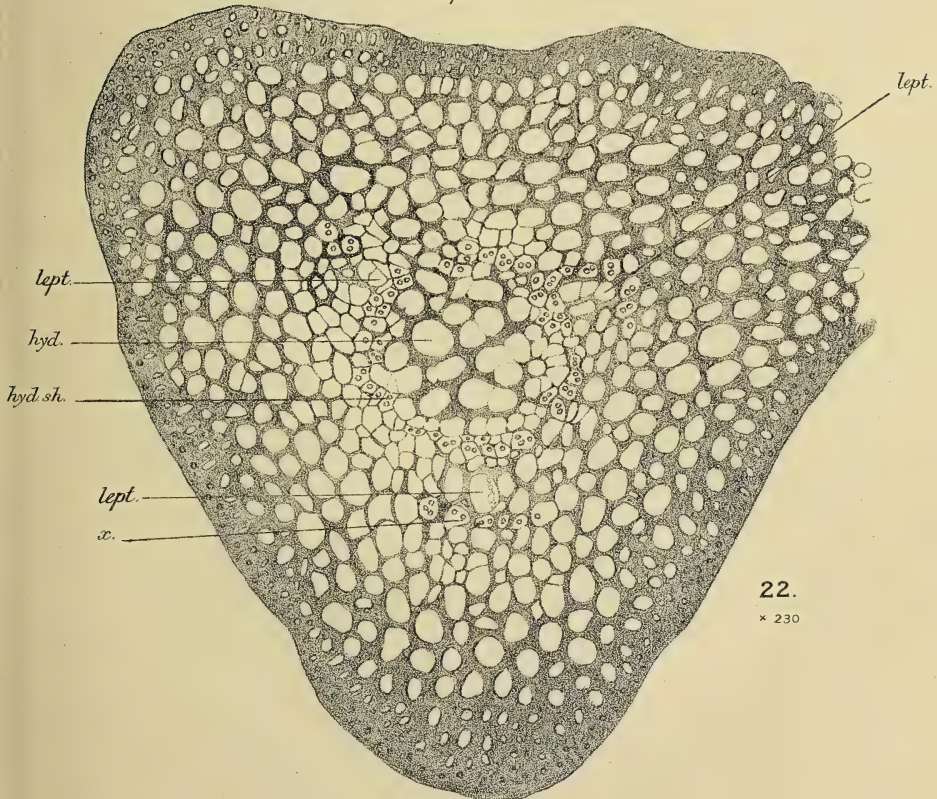
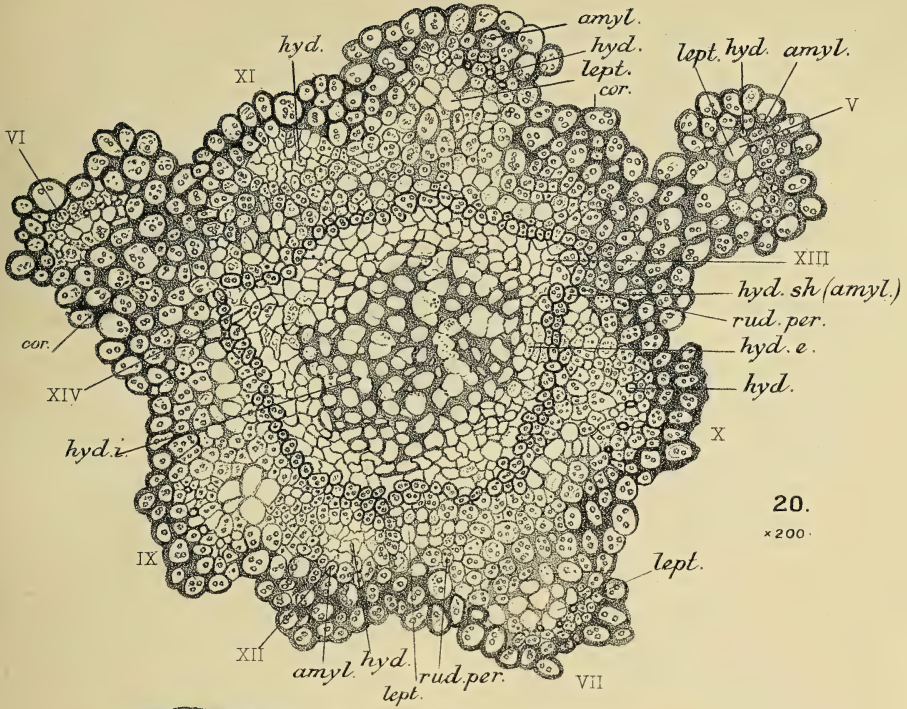


21.
 $\times 200$



19.
 $\times 270$

VIII





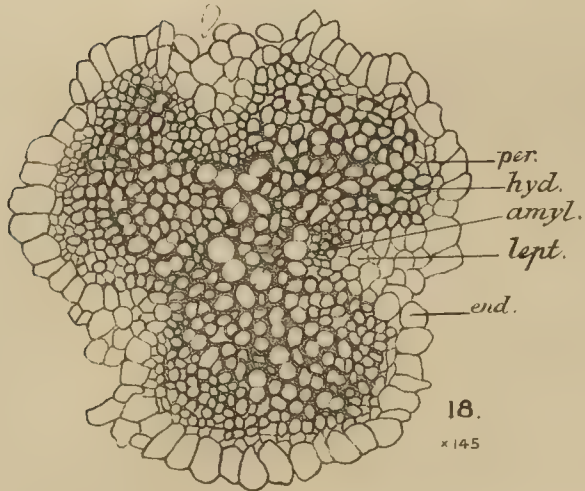
16.
× 145



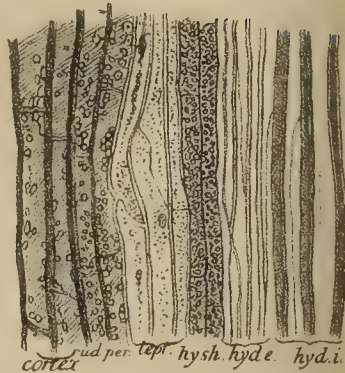
16a.
× 570



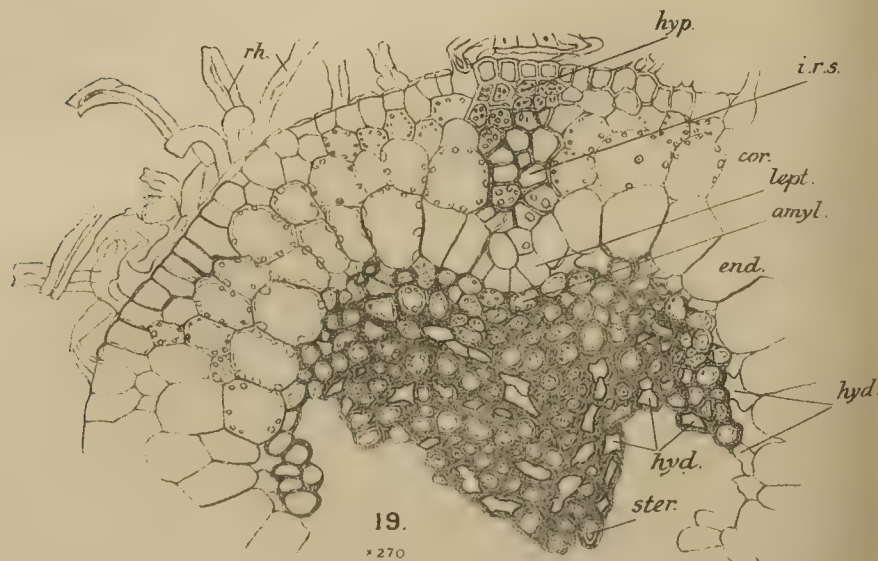
17.
× 385



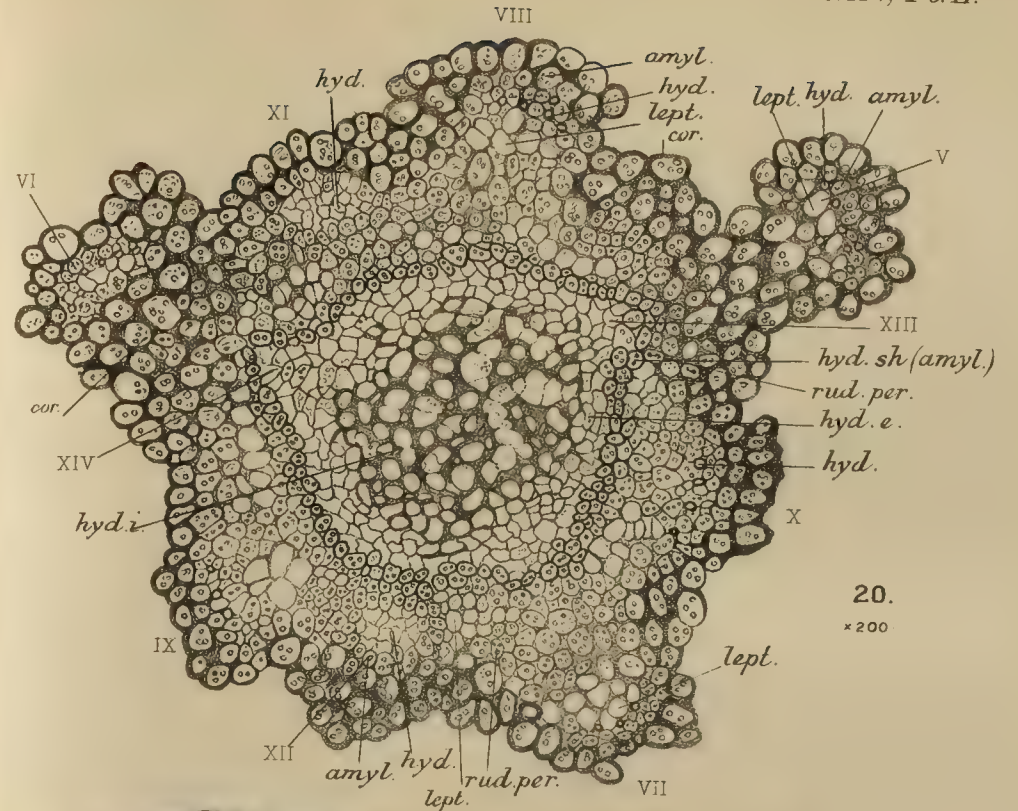
18.
× 145



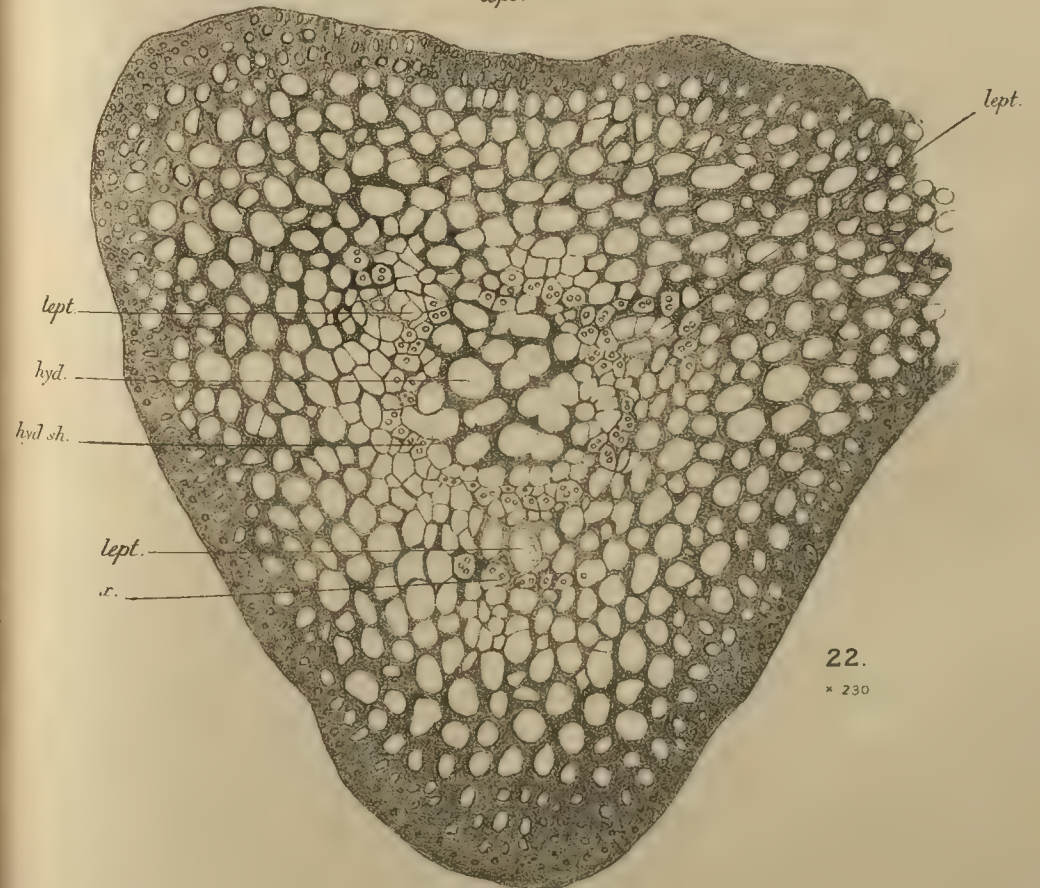
21.
× 200



19.
× 270



20.
× 200



22.
× 230

On the Effect of Salts on the Assimilation of Carbon Dioxide in *Ulva latissima*, L.

BY

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SECTION I. METHOD.

THE primary object of this research was to obtain some idea of the extent to which the power of carbon-assimilation is dependent on the absorption of nutrient salts, and of the inhibition caused by the presence or absence of certain salts in the medium. The experiments were intended to be primarily qualitative rather than quantitative, and the standpoint throughout was the power of carbon-assimilation, and not growth or development.

For this purpose a plant possessing a minimum of specialization in regard to both carbon-assimilation and absorption was best suited. Certain marine Chlorophyceae present an additional advantage in that they grow in a medium containing an exceedingly constant proportion of dissolved salts. The Alga used throughout this work was *Ulva latissima*, Linn. *Enteromorpha intestinalis*, Link., was also tried, but presented difficulties which were for the most part absent in the case of *Ulva*, and for this reason was early abandoned.

The method employed was to obtain *Ulva* free from starch, and then to expose it to light for various periods in solutions of known composition. The amount of starch formed in each

case was taken as a measure of the carbon-assimilation during the period of exposure.

The *Ulva* I used was partly obtained from the Welsh coast at Bangor through the kindness of Professor Phillips, and partly from Hunstanton on the Norfolk coast. I found that the *Alga* succeeded best when gathered as near the low tide limit as possible. Another point of great importance was that it should be free from other kinds of seaweed. The *Ulva* was placed in sea water in large earthenware vessels, in a cool dark room in the laboratory. The temperature of this room was at the end of April about 16° – 18° C. at midday; at the end of June between 18° – 20° C., or even higher. The sea water was supplied by the Great Eastern Railway from the coast at Lowestoft. The specific gravity of two samples was taken and found to be (1) 1.026; (2) 1.0254. The average specific gravity of sea water is about 1.027. The samples were therefore slightly lower than they should have been, but the difference is so small as to be negligible.

The chief difficulty experienced in this work was to keep the *Alga* in good condition during the prolonged period of darkening that was necessary to get rid of all accumulated starch. During the winter and spring this difficulty was not so great, but in May and June it was found to be almost impossible to preserve the *Algae* in a healthy state, although the period of darkening was then shorter. This difficulty was undoubtedly one of temperature. Modern work¹ has shown that these *Algae* require a comparatively low temperature; in fact, as cool a situation as possible. I had no means at my command for keeping down the temperature in

¹ During the last decade considerable attention has been directed to the best physical conditions and mediums in which to cultivate marine and freshwater *Algae*. Attention may be called to the following works:—Klebs ('96), Oltmanns ('92 and '95), Noll ('92), Ward ('99), Molisch ('95). References to the works quoted will be found in the Bibliography at p. 69, at the end of this paper. The numbers in brackets after the authors' names indicate the year of publication of the work referred to in the Bibliography. Thus Noll ('92) means that the paper was published in 1892.

any way for a long period, or for the large amount of material which was necessary for the work. Another difficulty, which also was no doubt partly due to the difficulty with temperature, was the enormous increase of Bacteria during the period of darkening. These Bacteria also make their appearance, but in a less degree, in cultures exposed to light, as has been recorded by Benecke¹ and Oltmanns². In darkness, however, this becomes a matter of great difficulty. In less than a week, a most evil-smelling scum, white or brick-red in colour, and of appreciable thickness, makes its appearance on the surface. It is of course impossible to sterilize the sea water, and the only thing to be done is to constantly change the water at least three times a fortnight, and to skim the surface every other day. When the sea water was changed the Algae were rinsed thoroughly with tap water and drained in a sieve, and then put into a fresh basin of sea water. That the formation of the scum was largely due to the temperature, was proved by the fact that in June it was much greater than in March. It may be pointed out, as indeed Oltmanns, Noll, and others have shown, that the changing of the sea water is an absolute necessity in other ways, as by so doing a fresh supply of nutrient salts is brought within the reach of the plant. Another difficulty was found to be very common in cultures carried out in the summer months. The Alga seemed then to be more liable to lose its chlorophyll than in the spring, probably owing to the greater intensity and duration of the illumination on exposure in the greenhouse. On the whole, winter work, with longer darkening and less constant illumination, was found to give better results. In consequence of these difficulties I was unable in some cases to make as many experiments or to extend the work as far as I could have wished.

It was found to be fairly easy to tell, as a rule, when *Ulva* was in an unhealthy condition. Under adverse circumstances the Alga would become full of holes (as may be seen naturally

¹ Benecke ('98).

² Oltmanns ('95).

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in old specimens), and the thallus would fragmentate into small pieces, or the solution become cloudy. Side by side with this the chlorophyll would disappear; although this sometimes took place before fragmentation.

The period of darkening required to obtain the *Ulva* starch-free was much greater than was at first anticipated. In the winter and spring months a period of five weeks or longer had to be allowed. After a month, however, most of the starch had disappeared. In May and June the period was shorter; no doubt as a result of the higher mean temperature. In three weeks to a month all the starch had disappeared. The Alga was tested from time to time, and if after two or three testings no starch was found, it was at once used for exposure experiments.

Experiment I. *Ulva* darkened Feb. 24, 1900.

<i>Date.</i>	<i>Days.</i>	<i>Amount of Starch.</i>
Feb. 26	2	maximum
Mar. 10	14	large
Mar. 18	22	moderate
Apr. 7	42	a trace
Apr. 9	44	none or the slightest trace
Apr. 11	46	none
Apr. 15	50	none

Even after such prolonged darkening it was sometimes found that a small part of the thallus still retained a trace. A fact which was noticed throughout, and which I am unable to explain, was that when only a trace of starch was present, either as a residuum of darkening or as a minimum of carbon-assimilation, such starch was almost invariably to be found only at the edges of the thallus, and in this position there was in nearly all cases more starch than in the centre even when a moderate amount was present.

Another point observed in connexion with the darkening was the change in colour of the thallus when exposed to light

after darkening for some time. In the latter case the Alga was a dull, fairly opaque, dark green. On illumination of even a day it became much lighter in colour as well as more transparent—a brighter green generally. Harvey¹ has made the same remark about specimens of *Ulva* dredged from deep water, when 'the colour is of a very dark and even bluish green reflecting glaucous tints when under water.'

Enteromorpha intestinalis, Link., required an even longer period of darkening. In the spring not less than two months was found necessary, and even then one could not always be sure that every trace of starch had been got rid of. It was also found to be much more difficult to judge accurately the amount of starch in this Alga as compared with *Ulva*, and for these reasons it was early abandoned.

The method of testing for starch used was Sachs's iodine reaction². *Ulva* when in a healthy state decolorized easily, after being placed in boiling water for a minute or two, and then allowed to stand for a day in methylated spirit. A sufficient strength of watery alcoholic solution of iodine was used without perceptibly colouring the thallus yellow. In a few cases Schimper's chloral hydrate method was used as a confirmatory test. Sachs's iodine method with this Alga is, however, sufficiently delicate, and this plant had the great advantage that, by this method, the distribution of the starch over a considerable area of thallus could be estimated at a glance. The drawback with regard to *Ulva* consists in the extremely small size of the cells even under the ordinary high power ($\frac{1}{8}$ inch objective) of the microscope. Experiments with plasmolysis were not attempted, and they would be very difficult if not impossible for this reason. As might be expected, the appearance of the chloroplast was, as a rule, no guide to the condition, satisfactory or otherwise, of the Alga.

When the Alga had been rendered starch-free, it was exposed to light continuously in a cool greenhouse, which

¹ Harvey ('46), vol. 4, under *Ulva latissima*.

² Sachs ('88), p. 1.

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was kept carefully shaded in summer. The medium, or solution of salts, was contained in glass dishes holding about 700 c.c., and about $2\frac{1}{2}$ inches deep and 5 inches in diameter. The members of a series of experiments with different degrees of concentration of a salt and comparative experiments, were exposed at the same time and for the same length of time, so that the temperature and illumination were constant.

Before the Algae were placed in these solutions they were thoroughly rinsed several times with tap water and finally soaked once or twice for a few minutes in distilled water to get rid, as far as possible, of all traces of the salts of sea water. A preliminary series of experiments was made in order to make sure that this washing, especially the use of distilled water, did not exercise a prejudicial effect on the Algae. As will be seen from Experiment II, this was not found to be the case.

Experiment II. Starch-free *Ulva* washed in

(1) Tap water, and then (2) Distilled water.		Tap water only.	Control in sea water direct.
Days.	Amount of Starch.		
$1\frac{1}{3}$	moderate	moderate	moderate
3	moderate-large	moderate	moderate-large
5	large	large	moderate

This result was confirmed by other experiments.

Special mention must be made of the way in which the amount of starch formed under different conditions was compared. It was found easy to judge this by the eye, by paying attention to the distribution and the intensity of the colour obtained by the iodine reaction. I used an arbitrary scale of five degrees as follows :—

1. *Maximum*: thallus a uniform deep black.
2. *Large*: thallus black but starch not uniformly distributed, i. e. darker in some places than others.
3. *Moderate*: thallus bluish-black at the edges. Centre of the thallus blue, with less starch.
4. *Little*: thallus bluish at the edges with hardly a trace in the centre.
5. *Trace*: thallus only very faintly blue.

The following experiment, and the inferences drawn from it, may serve to illustrate the use of the starch-scale, and its limitations in regard to reliable evidence.

Experiment III. *Ulva* (starch-free) in sea water,
January 19, 1900.

Date.	Days' Exposure.	Amount of Starch.
Jan. 20	1	a trace
Jan. 23	4	a little
Jan. 29	10	moderate
Feb. 2	14	large
Feb. 6	18	maximum

It will be noticed that the amount of starch increased for some time until the maximum was reached. Such a gradual increase was in greater or less degree common to all experiments under conditions where carbon-assimilation could take place freely, and is readily explained. At the end of the first day's illumination the amount of starch in the cells is that formed during the day. But after n days' exposure the amount of starch in the thallus is that formed during the n^{th} day, and what remains of the starch formed during $n-1$ days. Consequently the iodine reaction after n days gives a much deeper coloration, the 'maximum' of the starch-scale representing the maximum of starch-accumulation. I wish to specially emphasize the fact that Experiment III was not made with the intention of finding the time necessary for the formation of a 'moderate' or any other degree of starch. The testing was only performed on the days indicated, and not

every day or at shorter intervals as would have been the case with such an object in view. The time required to reach any special point in the scale must obviously depend on external conditions, such as continued brightness of illumination, temperature, &c., as well as on the vitality of the plant itself. The effect of these is seen in a comparison between Experiments II and III, the latter being performed in the depth of winter and the former at the end of April. The object of such an experiment as No. III was to show that *Ulva* in sea water can assimilate freely, and that a maximum of assimilation is possible. This was the only kind of inference intended to be drawn from such experiments, and for this purpose the starch-scale given above was found to be quite sufficient.

The work was carried on during the past year in the University Botanical Laboratory at Cambridge. I may here take the opportunity of expressing my great indebtedness to Mr. Francis Darwin, who first suggested the subject to me, and who has helped me throughout by suggestions as to scope and methods. I am also particularly indebted to him for help with a somewhat scattered literature.

SECTION II. DISTILLED WATER.

Experiments with distilled water were especially interesting, as in such a medium there is of course a complete absence of nutrient salts. I found that it was only possible to keep *Ulva* alive for a short time in distilled water. In the majority of cases, a certain amount of starch was formed, although the amount was never 'moderate.' Out of nine series of experiments, five showed a 'little' starch. In two others only a 'trace' of starch, while in the remaining two, no starch could be detected even after several testings.

The amount of starch in the control in sea water on the seventh day was 'large,' showing that the Alga was in good condition originally. The Alga used was darkened on March 24, 1900, nearly two months before the experiment, and had been proved starch-free by repeated testings.

Experiment IV. April 19, 1900. Starch-free *Ulva* in distilled water.

Date.	Days.	Amount of Starch.
Apr. 20	1	a trace
Apr. 21	2	a little
Apr. 22	3	"
Apr. 23	4	"
Apr. 24	5	"
Apr. 25	6	"
Apr. 26	7	"

Usually after a week or even less in distilled water the Alga became unhealthy; the solution would become cloudy with Bacteria and the Alga fragmentate. I cannot but conclude, however, that this result was not due directly to the distilled water. Naegeli and others have found that distilled water has a poisonous or an injurious, if not fatal, action on plants. Naegeli¹ attributed this to the presence of a trace of copper, and found that one part of copper in a thousand million parts of water was fatal to a *Spirogyra* filament. On the other hand, Klebs², Oltmanns³, Molisch⁴, and others, have made use of distilled water in connexion with the cultivation of Algae without any disastrous results. The distilled water used in this work was the ordinary distilled water as supplied to the laboratory and not re-distilled, and was made use of in all my experiments wherever sea water or tap water were not employed. The fact that, whenever a sufficient amount of nutrient salts was added to such water, there was always, with the exception of a very small margin of failures, a greater or less amount of carbon-assimilation—the amount of starch often reaching the maximum—seemed to show conclusively that the distilled water in itself had no injurious effect. In Experiments IX-X (p. 61) and XII-XIII (p. 64), the cultures were extended for fully a month.

¹ Naegeli; vide Pfeffer ('00), p. 221.

² Klebs ('96).

³ Oltmanns ('92).

⁴ Molisch ('95).

Another series of experiments, similar in nature to those above described, were made in which *Ulva* was suspended in damp air. Starch-free *Ulva* was carefully rinsed with distilled water to get rid of all traces of sea water, and then roughly dried by being gently pressed in filter paper. It was then placed on a dry earthenware plate covered with a large glass dish, and exposed to light in a greenhouse. After two days in the early part of June, rather more than a 'trace' of starch, i.e., almost a 'little,' was obtained in the two experiments made.

Another experiment was made in which the plate held some distilled water and the Alga was placed on a brass stand similar to those commonly used in the laboratory for holding slides. The *Ulva* was so arranged that it did not dip into the water in the plate and the whole was covered with a large glass dish as before. The *Ulva* was kept moist and the dish was covered with dew from the evaporation of the water. In five experiments out of six a similar result was obtained to that in the experiments last mentioned. The Alga did not seem to be able to survive such treatment for a longer period than two days. The results, however, agree very closely with those obtained when *Ulva* was immersed in distilled water.

The conclusion I arrived at from these experiments, was that the function of carbon-assimilation in *Ulva* was dependent on the presence of suitable inorganic salts in the medium. It seemed clear for the reasons already given that these results could not be attributed to any prejudicial effect of the distilled water as a medium, but that the inhibition of the carbon-assimilation was caused by the entire and continued absence of nutrient salts, just as much as it might have been by unsuitable physical conditions. As might be expected, a prolonged continuance of this condition was found to be fatal.

In most of the experiments with distilled water it was found that a small amount of carbon-assimilation was possible, but the amount of starch-accumulation was never 'moderate'

in quantity. For reasons more fully expressed in Section V, I do not attribute this to an incomplete removal of all traces of sea water from the *Ulva* at the beginning of the experiment. These results can only, I think, be accounted for either by supposing that the carbon-assimilation can go on for a very short time in the total absence of inorganic nutrient salts, or that the small starch-accumulation is due to the presence of reserves of such salts within the plant. I am inclined to favour the latter hypothesis, since there is evidence to show that such reserves are constantly present in the plant. An experiment of de Saussure¹, made more than a century ago, illustrates this fact. De Saussure placed some Peppermint plants with their roots in pure water, in a place exposed to air and light but sheltered from rain. After allowing them to vegetate for a period of two and a half months, he found that plants which originally weighed 100 parts had increased to 216 parts, and the total dry matter, originally 40.3, had become 62 parts. It is obvious then that at least one-third of the ash of the original plants was in excess, and constituted a reserve which was drawn on during the two and a half months of culture in pure water.

The lack of knowledge of the nature and amount of the various inorganic reserves available within the plant was a constantly present difficulty throughout this work, and in this instance prevents any definite conclusion. The fact that a small amount of starch can be formed in the absence of nutrient salts, in no way invalidates the conclusion that a total inhibition of the carbon-assimilation can be caused by the *continued* absence of all the inorganic salts obtained by means of absorption.

SECTION III. TAP WATER.

Several experiments were made in which 'starch-free' *Ulva* was exposed to light in ordinary tap water, and the difference between these cultures and those in distilled water was very striking, and forms a good illustration of the fact

¹ De Saussure; vide Johnson ('69), p. 175.

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'that from ordinary tap water containing mere traces of salts, plants may collect large quantities of non-volatilizable mineral constituents, and that, moreover, these constituents are absorbed in proportions altogether different from those obtaining in the water in question¹.' The Cambridge tap water contains about .036 per cent. of total solids, of which the largest components are $\text{CaCO}_3 = .0118$ per cent. and $\text{NaCl} = .0032$ per cent. A typical experiment was as follows:—

Experiment V. April 11, 1900. *Ulva* exposed in tap water.

<i>Date.</i>	<i>Days.</i>	<i>Amount of Starch.</i>
Apr. 13	2	moderate
Apr. 15	4	large

This result was confirmed by four other experiments, although in one or two cases I failed to obtain more than a 'little' starch. I concluded, therefore, that for some time at least there was no marked inhibition of carbon-assimilation when the medium was tap water. Experiments for a period longer than a week were not however successful; the *Alga* becoming unhealthy and finally dying. In none of these experiments did I obtain the maximum of starch-accumulation. The larger amount of starch formed in a medium of tap water, as compared with distilled water, can only I think be accounted for by the fact that the former does contain some nutrient substances in however small proportions; and that the plant can make use of these to such a degree that for some time there is little inhibition of the carbon-assimilatory function, or at least only a very partial inhibition. If this is the case, we have here a striking illustration of the extent of the influence of nutrient salts on the power of carbon-assimilation.

These experiments would seem to throw some light on

¹ Pfeffer ('00), pp. 120-1.

the biology of Algae. It is well known for instance that certain Algae flourish in the estuaries of large tidal rivers, in localities which at low tide are practically freshwater. These results with tap water would seem to show that even at low tide the carbon-assimilation is not inhibited but that it can go on even in very *dilute* solutions, until on the return of the tide the water becomes strongly saline again. It has been long known that some marine Algae can accommodate themselves to brackish water and ultimately to fresh water, if the process is gradual and prolonged. On the other hand, other Algae are obligate Halophytes, and will not thrive for any length of time in dilute solutions of nutrient salts. To which class *Ulva* belongs I am unable to speak positively or definitely, as I had not the time at my disposal for the long accommodatory experiments necessary to answer this question. Whatever evidence these experiments afford, seems rather in favour of the view that *Ulva* is more or less obligate, and in that case these experiments with tap water show that a habitat, which is exposed to *short* periods of almost fresh water conditions, would cause a very slight, if any, inhibition of the power of carbon-assimilation, even to obligate Algae.

SECTION IV. SEA WATER.

Many cultures of *Ulva* were made in sea water, chiefly as controls to other experiments, and as a rule there was no difficulty in obtaining the maximum of starch accumulation. A 'moderate' amount of starch was usually formed in a couple of days or even less of bright weather, whereas in the case of Experiment III, in mid-winter, the period was much longer. The chief use of such controls, apart from comparison with other cultures as to the amount of starch formed, was to make sure that at the beginning of the experiment the material was in a healthy condition.

Sea water being the natural medium in which such Algae live, contains all the salts necessary for its life and

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development. A study of the composition of sea water is therefore important in itself.

Two analyses of British sea water may be quoted. Analysis *A* was made by Thorpe and Moreton¹ of water collected from the Irish Sea in winter (Sp. Gr. 1.02721 at 0°). Analysis *B* is from water from the Bristol Channel (Sp. Gr. 1.0274 at 15.5° C.)².

The form of these analyses has been altered here in order to bring the salts into order of their percentages.

<i>A</i>			<i>B</i>
<i>In 1000 parts.</i>			<i>Per Cent.</i>
Water . . .	966.14054	=	96.614
1 NaCl . . .	26.43918	=	2.644
2 MgCl ₂ . . .	3.15083	=	.315
3 MgSO ₄ . . .	2.06608	=	.207
4 CaSO ₄ . . .	1.33158	=	.133
5 KCl . . .	0.74619	=	.075
6 MgBr ₂ . . .	0.07052	=	.007
7 CaCO ₃ . . .	0.04754	=	.005
FeCO ₃ . . .	0.00503		
Mg(NO ₃) ₂ . .	0.00207		
NH ₄ Cl . . .	0.00044		
Traces of MgCO ₃ , LiCl, SiO ₂			

In the present paper it is proposed to deal with the five principal salts in sea water; the three chlorides (NaCl, MgCl₂, and KCl), and the two sulphates (MgSO₄ and CaSO₄). In a future communication I shall hope to give some account of the effect on carbon-assimilation of those salts which occur only as traces or in very small amounts in sea water. A brief *résumé* of the chief results obtained from both these series of experiments was given before the British Association at the recent Bradford meeting³.

¹ Thorpe and Moreton; vide Thorpe, p. 141.

² Tilden ('89), p. 67.

³ Arber ('00).

SECTION V. SODIUM CHLORIDE.

From the analyses of sea water quoted in the preceding section, it will be seen that sodium chloride forms by far the largest percentage (2.6-2.7 per cent.) of any of the salts in sea water, and this quantity is at least seven times larger than the amount of magnesium chloride (0.3 per cent.), the next largest constituent. A large number of experiments were made in which sodium chloride was *alone* present in the media, and to these reference will first be made. Starch-free *Ulva* was exposed in solutions of various strengths of the purest sodium chloride obtainable, dissolved in either distilled water or tap water. In every case, out of about thirty cultures with different percentages, a certain amount of starch was always found in the cells. Among the first of these experiments were those in which the amount of Na Cl was the same as in sea water, i. e. 2.65 per cent. The results of two of the experiments, one with distilled water and the other with tap water as mediums, are as follows :—

Experiment VI. Feb. 2, 1900. *Ulva* in 2.65 per cent.
Na Cl in distilled water.

Date.	Days.	Amount of Starch.
Feb. 5	3	moderate
Feb. 11	9	large
Feb. 20	18	large

Experiment VII. April 15, 1900. *Ulva* in 2.5 per cent.
Na Cl in tap water.

Date.	Days.	Amount of Starch.
Apr. 16	1	moderate
Apr. 18	3	large
Apr. 20	5	large

Other similar experiments confirmed this. With regard to the duration of the experiments, eighteen days was the limit to which any of my cultures with Na Cl were carried, and in this time I have never observed the maximum of starch storage in cultures of this or any other percentage of sodium chloride *alone*. The results with tap-water solutions of Na Cl were very similar throughout to those with distilled water; if anything a little more starch was formed.

Experiments were also made to find the percentage of sodium chloride which gave the best results in the way of starch-formation, and the effect of dilute and concentrated solutions of the same salt. Lesage¹ is among the very few writers who have directly estimated the effect of the amount of salt (Na Cl) present in the soil on the power of starch-formation². Lesage found that the quantity of starch formed in the vegetative organs of *Lepidium sativum* was directly influenced by the amount of sodium chloride present. The maximum of starch-formation was reached when the plant was watered with a solution containing 2.5–5 grams Na Cl in a litre. The lower minimum was found to be 1.6 grams per litre; and the upper, 12.5 grams Na Cl in a litre, when no starch was formed. Lesage came to the general conclusion that the amount of starch decreases as the amount of sodium chloride increases, but not in the same degree. Richter³ found that many freshwater Algae can be accustomed to solutions of sodium chloride, provided the adaptation is not too forcible. Plasmolysis was never observed in experiments in which the increase in the percentage of the salt was gradual and spread over a considerable period. At the end of each successive adaptation, i.e. the point at which the plant became fully acclimatized, there was a marked increase in the amount of starch formed and stored, and this was

¹ Lesage ('91).

² A considerable amount of work has been done by Strange ('92), Janse ('87), Oltmanns ('95), and others on the effect of concentration of the medium, but the standpoint has been almost entirely that of growth and development.

³ Richter ('92), p. 55.

wholly or partly consumed during the next period of adaptation to a slightly stronger solution.

On the whole I found that *Ulva* was very sensitive to the amount of sodium chloride present in the solution. In a solution containing only 0.005 per cent. NaCl in distilled water, I obtained a 'moderate' amount of starch in six days in February, and a similar amount in 0.01 per cent. in a parallel culture. This was a larger amount of starch than I ever obtained in distilled water. Another culture in 0.1 per cent. under the same conditions and in the same time gave a 'large' amount of starch. It was found that the amount of starch formed in parallel cultures of the same duration increased up to a little beyond 2.5 per cent., and on the whole the largest amount of starch was obtained in solutions of this strength. It would be perhaps stretching the capacity of the starch-scale too far to say when the exact maximum was reached, a result which would depend to some extent, no doubt, on the vitality of the individual. As far as my experiments go I should be inclined to estimate the maximum of concentration as between one and five per cent. Experiments with higher percentages showed that the assimilation became smaller, as was expected from results of the observers already mentioned. The cause of this diminution is not very clear. It is true that plasmolysis may have taken place, a point which, from the difficulties which *Ulva* presented in the extreme smallness of its cells, I made no attempt to determine here or in other cases. But plasmolysis does not necessarily imply inhibition of carbon-assimilation, for it has been found that plasmolysed cells can assimilate. The effect is more probably due to the large quantity of the salt within the cells hindering the metabolism. The highest percentage of NaCl used was 7.5 per cent. in tap water, and, as the following experiment shows, the amount of starch was sensibly less.

Experiment VIII. April 15, 1900. *Ulva* in a solution of Na Cl in tap water.

<i>Date.</i>	<i>Days.</i>	2.5% Na Cl.	5% Na Cl.	7.5% Na Cl.
Apr. 16	1	moderate	little	trace
Apr. 18	3	large	moderate	little
Apr. 20	5	large	large	moderate

Any direct attempt to estimate *the effect of the total absence of sodium chloride*, and more especially of the element sodium, would necessitate an exceedingly difficult and laborious piece of research. For such, the most pedantically accurate experiments, as well as the most stringent precautions, would alone suffice. In the first place it would be exceedingly difficult to obtain *Ulva* free from starch and in good condition, without any trace of the sodium chloride from the sea water in which it was darkened. Glass vessels could not be used, and the water could not be distilled from glass retorts. Lastly, perhaps the most insuperable difficulty of all would be the air, which always contains sodium in the shape of dust. The complete absence of sodium would therefore be very difficult to insure. Pfeffer¹ says that 'seaweeds have not up to the present time² been cultivated in the absence of sodium chloride,' nor 'has it been found that plants frequenting saline habitats can exist without sodium.'

It was no part of my object to solve the problem of the *complete absence* of sodium chloride. I am able, however, to offer indirect evidence from experiments in which the amount of sodium chloride was apparently only very small, if any at all was present. I may here anticipate the results obtained from the experiments in which a single one of the principal salts in sea water, other than Na Cl, was alone present in the medium. A fuller account of these experiments is contained

¹ Pfeffer ('00), p. 411.² 1897.

in the two following sections. In these it is possible that in all cases a small amount of Na Cl may have been present as a residuum of the original sea water. This amount, however, seems to have been quite negligible, for the following reasons.

As already stated, I found that *Ulva* in 0.005 per cent. Na Cl in distilled water gave a 'moderate' amount of starch in a week at the end of April. In experiments with K Cl alone (vide section 6), in which the percentage in distilled water was 0.07 (the amount in sea water), I was unable to obtain more than a 'trace' of starch. Now, if the impurity of Na Cl present were as large as 0.005 per cent., there would seem to be no reason why a 'moderate' amount of starch would not have been formed, despite the presence of 0.07 per cent. K Cl; for the element potassium is absolutely essential to the plant. Further, in no case in which a single one of the salts $MgCl_2$, $MgSO_4$, $CaSO_4$, and K Cl was used alone, in any percentage that I experimented with, did I obtain a 'moderate' amount of starch. The small amount of starch found might be accounted for in some cases through the medium being too dilute, but if K Cl were as favourable to carbon-assimilation as Na Cl, a solution of 2 per cent. K Cl, which is equivalent to about 1.6 per cent. Na Cl, should have given a 'large' amount of starch within a week, as was found with this percentage of the latter salt; whereas only a 'trace' was obtained. It would therefore seem that, if these results with chlorides and sulphates of potassium, magnesium, and calcium hold good, the amount of Na Cl as an impurity is so small as to be negligible, in fact, that it may be *considered as absent altogether*. The lack of even a moderate amount of starch-accumulation, which was characteristic of experiments with solutions of these salts, cannot, I think, be in any way due to the starch being used up for growth, as in nearly all cases, especially in solutions of $CaSO_4$ and K Cl, the plants become obviously unhealthy after a short time.

Conclusions.

The general result of these experiments was that sodium chloride, in the absence of other salts, is highly favourable to carbon-assimilation, and the presence of this salt alone in the medium, if in sufficient degree of concentration, can induce a considerable amount, but not quite the maximum, of starch-storage. On the other hand, an almost complete absence of sodium chloride was found to cause a marked inhibition of the carbon-assimilation. It would appear, therefore, that sodium chloride is a salt indispensable to *Ulva*, and perhaps to other marine Algae, for the maintenance of carbon-assimilation.

The physiological explanation, which is provisionally suggested here, is that sodium chloride is a food-substance essential to the metabolism of the Alga. It is not of course necessary, as Richards¹ has recently pointed out, that a substance which by its presence acts as a stimulus to growth or carbon-assimilation should be actually used as a food-material by the plant. There is, however, some evidence from recent research for the view that sodium chloride plays a more essential part in the economy of these plants than that of a mere stimulus. It is an undeniable conclusion, which has served as the starting-point for the researches of Schimper and others, that the cell-sap in such plants is never entirely free from this salt. Diels², in a recent paper, has shown experimentally, that when a Halophyte is cultivated in distilled water there is a removal of the contained sodium chloride from the tissues. By estimating the quantity of Na Cl in the ash, he found that 5 grams of the leaf of *Cakile maritima*, Scop., growing on the outer sand-dunes of the coast, contained 1.2 per cent. Na Cl. After 5 days' cultivation in distilled water, 5 grams of the leaf contained only .39 per cent. Na Cl. These results were confirmed by experiments on *Salicornia herbacea*, L. In one case the diminution in the

¹ Richards ('97), p. 676.

² Diels ('98).

amount of Na Cl during a week's cultivation in distilled water was 21.87 per cent. Diels concludes that the chloride is decomposed within the plant, especially when the Na Cl in the cell-sap tends to rise beyond a certain definite degree of concentration. He connects the decomposition of the chloride with the free organic acids, which Kraus¹ and others have shown to be present in Halophytes; probably as a result of restricted gaseous exchange, and the defective combustion of the carbohydrate. In the Halophytes which Diels experimented with (*Cakile maritima*, Scop., *Arenaria (Honckenya) peploides*, L. &c.), malic acid was found to be present; and Diels concluded that possibly the sodium resulting from the decomposition of Na Cl united with the organic acid, and that sodium malate was formed in the first place from the decomposition of the chloride. This latter conclusion is admittedly conjectural, and not founded on experimental evidence. With regard to the chlorine set free from the Na Cl, Diels concludes that it unites with the hydrogen of the organic acid, and is returned to the exterior by secretion of the roots. This would seem to be a most remarkable statement, and can only be taken, I think, as showing that Diels was unable in any way to trace the ultimate fate of the chlorine.

Hansteen² has concluded that Na Cl, and also K Cl, stand in a definite relation to the formation of proteids from amides and carbohydrates. If Na Cl is present in a cell capable of proteid-formation and of carbon-assimilation, one of two things would seem to happen. In the one case, the presence of salt tends to prevent the glucose reacting with the amide, and the cell remains poor in proteids but rich in carbohydrates. The other case seems to present reverse conditions; an abnormal acceleration of proteid being formed at the expense of the carbohydrate. Hansteen thinks that it depends on the quantity of the chloride present which reaction takes place.

In the case of proteid formation it is probable, therefore,

¹ Kraus ('86).

² Hansteen ('96).

that sodium chloride acts as a controlling agent, but I am inclined to think that the large amount of the removal of Na Cl from the leaf, as found by Diels, and the extreme importance of this salt as shown here, are strongly in favour of the view that in such Halophytes as *Ulva* the Na Cl is also essential for the metabolism. Whether it is the acid or the base, or both, which is necessary, is a question which only further work can answer.

Lastly it is interesting to contrast the results obtained with *Ulva* with the conclusions which have been drawn from non-halophytic Phanerogams. Schimper¹ has concluded that for the great majority of plants Na Cl is of no importance as a food material, and further that in salt-solutions the assimilation is inhibited to such a degree that starch and sugar are no longer produced. Stahl², Lesage³, and Dassonville⁴, among others, have also concluded that salt-solutions tend to injure or diminish the carbon-assimilatory apparatus. Richter⁵ has however pointed out that these results do not necessarily hold in the case of freshwater Algae.

SECTION VI. OTHER CHLORIDES IN SEA WATER.

Besides Na Cl, two other chlorides—magnesium chloride and potassium chloride—occur in sea water in the proportion of 0.3 per cent. and 0.07 per cent. respectively. Potassium is an essential element to all plants, and in case of *Ulva* is derived entirely from the small proportion of KCl in sea water. Magnesium is also essential, and is obtained from the chloride, sulphate, or the very small amount of nitrate in sea water. Experiments were made in which *Ulva* was exposed in a solution made up of distilled water and all the principal salts of sea water in the proportions which they there occur, with the exception of Mg Cl₂ in one case, and K Cl in the other. The absence of either salt did not seem to affect the amount of starch formed in any way.

¹ Schimper ('98), p. 98, and ('91), p. 26.

² Lesage ('90).

⁴ Dassonville ('96).

³ Stahl ('94), p. 136.

⁵ Richter ('92).

Experiment IX. Artificial sea water without Mg Cl_2 .

Begun Jan. 19, 1900.		
<i>Date.</i>	<i>Days.</i>	<i>Amount of Starch.</i>
Jan. 20	1	trace
Jan. 23	4	moderate
Jan. 29	10	large
Begun Jan. 30, 1900.		
Feb. 14	14	maximum
Mar. 1	29	maximum

Experiment X. Artificial sea water without K Cl.

Begun Jan. 19, 1900.		
<i>Date.</i>	<i>Days.</i>	<i>Amount of Starch.</i>
Jan. 20	1	moderate
Jan. 23	4	trace
Jan. 29	10	moderate
Begun Jan. 30, 1900.		
Feb. 14	14	maximum
Mar. 1	29	maximum

Experiment XI. Control. Natural sea water.

Begun Jan. 19, 1900.		
<i>Date.</i>	<i>Days.</i>	<i>Amount of Starch.</i>
Jan. 20	1	trace
Jan. 23	4	little
Jan. 29	10	moderate
Begun Jan. 30, 1900.		
Feb. 14	14	maximum
Mar. 1	29	maximum

The conclusion which I came to was that the absence of normal amounts of Mg Cl_2 or K Cl did not have any appreciable effect, and that these salts *alone* would not have a very great influence in maintaining the normal amount of carbon-assimilation. In Exp. IX, sufficient magnesium was probably present for the needs of the plant in the magnesium sulphate, and from Exp. X, I gathered that the plant could manage for some little time without access to potassium.

Experiments were also made in which *Ulva* was cultivated in various percentages of Mg Cl_2 and K Cl , all other salts being, as far as possible, absent.

(A) Mg Cl_2 .

This series of experiments was unfortunately left until the summer was advanced, and for this reason I was not able to carry them as far as I could have wished, and the deductions which I have drawn must be regarded as provisional. Solutions were tried which contained 0.31 per cent. (the amount in sea water), 1 per cent. and 2 per cent. Mg Cl_2 made up with distilled water. Two series of each of these percentages gave, after repeated testings, either a 'trace' or a 'little,' the same amount which would have been obtained in distilled water alone. In no case was a 'moderate' amount obtained; and after a short time the Alga did not appear to be in a very flourishing condition.

(B) K Cl .

In the case of this salt a larger number of experiments were made, and under more favourable conditions than with Mg Cl_2 . Solutions of 0.07 per cent., .5 per cent., 1 per cent., and 2 per cent. K Cl were used. They all gave similar results. Either the amount of starch was nil, or a 'trace' only was found. In one instance the amount was almost a 'little,' which was the largest degree of starch I could obtain. In less than a week the Alga usually became obviously unhealthy.

The conclusion with regard to these chlorides (provisional in the case of Mg Cl_2) was that the presence of either salt by itself in any percentage tried was not favourable to the maximum, or even to a moderate degree of carbon-assimilation. In the case of K Cl the amount of starch was even smaller in many cases than that which would have been formed in distilled water alone.

(C) Na Br .

A few experiments were made with sodium bromide to see whether this salt could take the place of the similar salt Na Cl . The concentrations used were 1, 2, and 2.5 per cent. Na Br . Six experiments with these percentages all gave very similar results. Some starch was formed in all cases, but a 'little' was the largest amount obtained after repeated testings for a week or more. Bromides are known to be non-injurious if sufficiently dilute, but as far as these experiments go Na Br did not seem able to take the place of Na Cl in regard to carbon-assimilation.

SECTION VII. SULPHATES.

The two sulphates which occur in sea water are Mg SO_4 (0.2 per cent.), and Ca SO_4 (0.1 per cent.). Calcium, and probably also magnesium, are essential in the case of the higher Algae, as has been shown by the recent researches of Molisch¹, Klebs², Benecke³, and Loew⁴.

Experiments were made with an artificial sea water, in which all the principal salts were present in the usual proportions, with the exception of magnesium sulphate in the one case, and calcium sulphate in the other. Distilled water was used as before and the *Ulva* freed as far as possible from sea water beforehand. In neither case did the absence of these salts seem to affect the carbon-assimilation.

¹ Molisch ('95).

³ Benecke ('98).

² Klebs ('96).

⁴ Loew ('98).

Experiment XII. Artificial sea water without Mg SO_4 .

Begun Jan. 19, 1900.		
<i>Date.</i>	<i>Days.</i>	<i>Amount of Starch.</i>
Jan. 20	1	moderate
Jan. 23	4	moderate
Jan. 29	10	¹
Begun Jan. 30, 1900.		
Feb. 14	14	maximum
Mar. 1	29	maximum

Experiment XIII. Artificial sea water without Ca SO_4 .

Begun Jan. 19, 1900.		
<i>Date.</i>	<i>Days.</i>	<i>Amount of Starch.</i>
Jan. 20	1	moderate
Jan. 23	4	moderate
Jan. 29	10	²
Begun Jan. 30, 1900.		
Feb. 14	14	maximum
Mar. 1	29	maximum

The control in sea water was the same as in Experiment XI, and in nearly all cases the amount of starch formed during these experiments was fully equal to the control. *Enteromorpha* gave very similar results. In the first experiment sufficient magnesium was available from the Mg Cl_2 present, and in both a sulphate was present. The only special point of interest was that the absence of calcium in

¹ *Ulva* was not estimated here, but *Enteromorpha* gave a moderate account.

² *Enteromorpha* moderate.

Experiment XIII did not seem to affect the amount of carbon-assimilation. Whether the plant can get on without it for a time if magnesium is present, or whether reserves of calcium exist in the plant, did not fall within the province of this paper to determine. Perhaps a very little calcium only is needed, and this may have crept in as an impurity in these relatively rough experiments. The conclusion drawn, however, was that the absence of either of these salts did not markedly affect the power of carbon-assimilation.

As in the case of the chlorides, experiments were made with various percentages of these salts *alone*.

(A) Ca SO_4 .

Two series of experiments were made; in one the amount was the same as in sea water ($\cdot 14$ per cent.), and the other was a saturated solution. In the latter case, 5 grams Ca SO_4 , finely powdered, were placed in 500 c.c. distilled water, and constantly stirred. After allowing the solution to stand for some hours, the undissolved Ca SO_4 was filtered off. The solution, therefore, contained considerably less than 1 per cent. Ca SO_4 . Starch-free *Ulva* was allowed to remain for 8 days or more in these two solutions, but after frequent testings, with two series of experiments, I never obtained more than a 'trace,' while in the great majority of cases no starch whatever was found. The *Ulva* began to fragment, and show other signs of becoming unhealthy after a few days. These experiments were conducted in the latter part of June, when, as already explained, the conditions were unfavourable. The conclusion which, I think, may be provisionally drawn is that Ca SO_4 alone cannot take the place of Na Cl in regard to carbon-assimilation.

(B) Mg SO_4 .

Magnesium sulphate is readily soluble in water. Solutions containing 0.23 per cent. of this salt in distilled water (the amount in sea water) gave in all cases a 'trace' or a 'little'

starch. In experiments with 1 or 2 per cent. Mg SO_4 I failed to get a larger amount of starch. I therefore concluded that Mg SO_4 was a salt less favourable to carbon-assimilation than Na Cl .

(C) Na_2SO_4 .

Glauber's salt does not occur in sea water, but two experiments were tried in the hope that a larger amount of starch would be obtained than in the case of the other sulphates. A 2 per cent. solution was used, and gave similar results; after 3 days a 'trace,' after 7 days a 'little.'

The general conclusion of these experiments with sulphates alone was similar to those obtained with chlorides other than Na Cl . The amount of starch was never greater than a 'little,' often only a 'trace,' a result which would have been obtained by using distilled water alone. The experiments with Ca SO_4 seemed to give even a worse result.

SECTION XIII. CONCLUSIONS.

There has been a tendency in recent work to call greater attention to the importance of the absorption of inorganic salts for the maintenance of carbon-assimilation. Stahl¹ in particular has adopted this standpoint in regard to the theory of the biology of nyctitropism.

From my experiments it seems clear that in the case of *Ulva* a marked inhibition of the power of carbon-assimilation can be caused by the absence of suitable or necessary inorganic salts, and especially by an almost complete absence of sodium chloride. The absence of a certain salt from the medium may cause an inhibition just as much as the presence of an unfavourable salt in the medium. In *Ulva*, and probably in other plants of similar structure and habitat, sodium chloride was found to have a peculiar value in regard to carbon-assimilation, and although a full measure of carbon-assimilation is doubtless only reached when the whole or

¹ Stahl ('97), p. 82, &c.

most of the other salts in sea water are present, none of these salts could be found to replace sodium chloride in this respect¹. In most of the experiments in which an inhibition was caused by the presence or absence of a certain salt, such an inhibition was rarely absolute at first. Even in experiments with distilled water, a very small amount of starch was detected, and this may have been due to reserves of inorganic material within the plant. If these conditions were continued, not only did the inhibition become absolute, but such conditions were generally found to be fatal to the plant.

Ulva is a member of a special biological group of plants, the Halophytes, and probably an obligate Halophyte. In such no doubt there are special requirements which may have in part arisen as adaptations to the environment, and which are largely or totally absent in other groups of plants. Consequently such conclusions as are here drawn are not general, but only applicable to such plants as are Halophytes, and perhaps only to obligate Halophytes.

The following is a short summary of the chief results obtained:—

1. Distilled water for a short time allows of a very small amount of carbon-assimilation, but is quickly fatal through the absence of all essential inorganic salts.

2. Tap water, containing only a small percentage of salts, will permit of a comparatively large amount of carbon-assimilation, but not the maximum.

3. Sea water, being the natural medium of these plants, allows of the maximum of carbon-assimilation within the shortest time.

4. Sodium chloride seems to be an absolutely indispensable salt to the medium for even a moderate amount of carbon-assimilation. From indirect evidence, in which this salt was as far as possible absent, in rough experiments, there was found to be a marked inhibition.

¹ It is possible that by a process of accommodation $MgCl_2$, or some other salt, could be made to take the place of $NaCl$, but this is a point which it did not fall within the province of this work to determine.

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5. The maximum degree of concentration of sodium chloride is probably between 1 and 5 per cent.

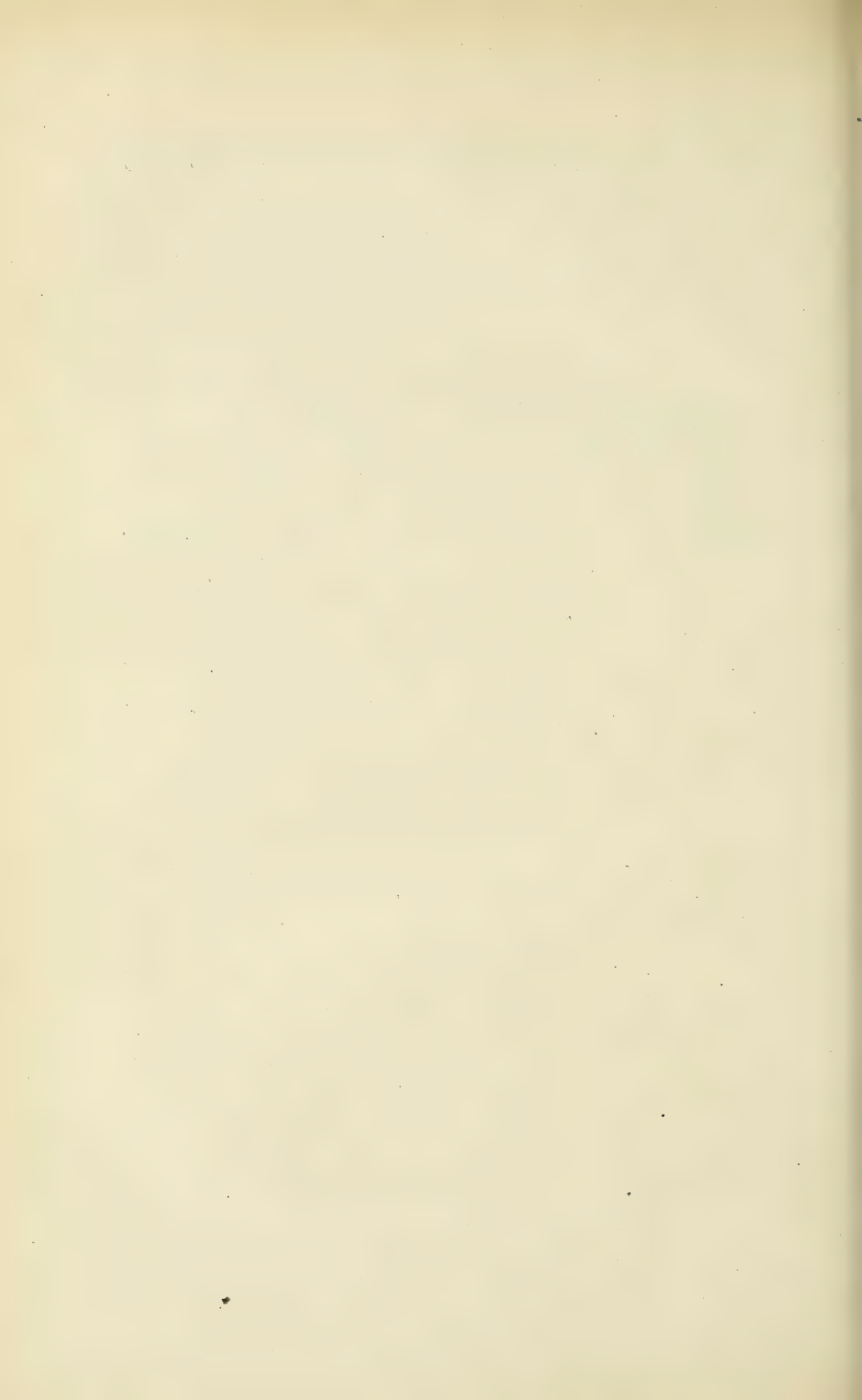
6. None of the other salts which form the principal constituents of sea water can take the place of Na Cl in any degree of concentration experimented with. The same remark applies to Na Br, Na_2SO_4 .

7. The absence of any one of the principal salts in sea water other than sodium chloride, provided the others were present in the normal amounts, did not cause any inhibition.

8. The presence of either CaSO_4 or K Cl in distilled water seemed to inhibit the carbon-assimilation almost completely, especially when in greater percentage than the normal amounts in sea water.

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Observations on the Anatomy of Solenostelic Ferns.

I. Loxsoma.

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With Plate III.



INTRODUCTION.

WHEN the idea of polystelic structure was first enunciated by Van Tieghem in 1886¹, he introduced at the same time the word *gamostely* in order to distinguish that particular type of polystelic structure which he regarded as arising by the more or less extensive fusion of originally separate steles. A small number of large curved plates or even a single closed tube may be formed as a result of this supposed fusion, and in continuance of this terminology each of these vascular masses is to be called a *gamostele*. In the second edition of his textbook, published in 1891², Van Tieghem offers another term, *solenostely*, as an alternative to *gamostely*, although he himself makes no further use of the suggestion, apparently preferring the original term, which he retains in all his subsequent works. Nevertheless, for the purposes of this

¹ Sur la Polystélie, Ann. des Sc. Nat. (Bot.), sér. vii, tome iii.

² Traité de Botanique (2nd ed.), p. 1372.

paper, so many advantages are possessed by the alternative term solenostely, that, instead of employing the older word, retained by Van Tieghem, it becomes clearly advisable to return to the rejected term. The most important of these advantages is the complete freedom from that suggestion of fusion that is implied by the word gamostely; and since, for the present at any rate, it is intended to use the term in a descriptive sense only, as a more convenient expression for the closed tubular bundle of De Bary¹, the absence of this idea is a very valuable property of the word. Indeed, it becomes an advantage of critical importance when it is considered that evidence is forthcoming which renders it extremely probable that in most cases no process of fusion whatever has anything to do with the production of a solenostele. As regards the Ferns in particular, it may be safely stated that in most cases the solenostelic arrangement of the vascular tissue is more primitive than the polystelic, since it has been shown in several Ferns that the final polystelic condition is *preceded* by a solenostelic stage at one time or another in the life-history of the individual plant. In such a plant the solenostele cannot be described as arising from the fusion of polystelic steles, but rather the polystelic state as brought about by the splitting up of a solenostelic ring. However, it is not to be inferred from this statement that no cases of real gamostely (in Van Tieghem's sense) exist at all; on the contrary, it does not seem by any means unlikely that such cases should occur, even among the Ferns themselves, and if future research should bring such a one to light, the word gamostely remains ready to hand as a term most suitable for the occasion. The fact, however, remains that gamostely, as at present applied, is a misleading term, because the structure indicated by it has been shown to be susceptible of an explanation exactly opposite to that implied by the term itself.

Again, the distinction between solenostely (gamostely) as defined by Van Tieghem, and *dialystely* (polystely with free

¹ Comp. Anat. (Engl. ed.), p. 284, 1884.

unfused steles) is merely one of degree, cases often arising in which it is no easy matter to decide which is the more suitable expression to use. Therefore, since the utility of a descriptive term will be the greater the more it is susceptible of accurate delimitation, it will be advisable to state at once that a solenostelic stem, as understood in this paper, may be defined as *one in which the vascular tissue is arranged in a single hollow cylinder with phloem and phloeotermia on either side, the complete continuity of which is interrupted only by the departure of the leaf-traces; the gaps thus produced being closed up in the internode above before the departure of the next leaf-trace.*

Whilst engaged in the preparation of this paper I was privileged to see an advanced copy of an exceedingly interesting and suggestive paper by Dr. Jeffrey¹, in which Van Tieghem's terminology is discussed at some length, and precisely the same opinion regarding the misleading nature of the term gamostely is arrived at (l.c., p. 605). Jeffrey has proposed an altogether new terminology based upon the word *siphonostele*, which, I take it, may be defined as a fibro-vascular tube interrupted by lacunae opposite the points of departure of branches, or of leaf-traces (l.c., p. 632). Although I am not yet prepared to accept all the phylogenetic relationships that are to be inferred from Jeffrey's system, in particular that relating to the Phanerogamic medullated monostele, yet many of the distinctions made are peculiarly fortunate, and coincide with structural features of crucial importance. In any case, no specific term is supplied for the particular vascular arrangement defined above as solenostelic, to which I believe it will be found convenient, in describing the anatomy of the Ferns, to give a name of its own. According to Jeffrey's terminology, solenostely would be regarded as a special type of *amphiphloic phyllosiphony*.

Although the solenostelic type of vascular structure is in general one of very rare occurrence, still a fair number of

¹ The Morphology of the central Cylinder in the Angiosperms; Transactions of the Canadian Institute, vol. vi, p. 599.

excellent examples are to be found among the Ferns, more frequently perhaps than among any other class of plants. Finding that these plants have not yet been closely investigated with reference to this particular character, I have been occupied for some time in examining all those species presenting indications of solenostely that I have been able to obtain. In the course of these investigations some alcohol-material of *Loxsoma Cunninghamii*, R. Br., was placed at my disposal by Professor Bower, to whom it had been sent by Messrs. Thompson and Cheeseman from New Zealand.

Hardly anything has hitherto been written upon the anatomy of this rare and interesting Fern, and since it obtains additional importance both on account of its isolated systematic position and of its limited distribution, it would seem to deserve a somewhat detailed anatomical description. I have therefore ventured to devote a special part of this paper to that purpose, reserving for a second part the less detailed descriptions, and the more general results that may arise from the investigation of the other solenostelic Ferns that I have been able to examine.

The history of the nomenclature of *Loxsoma*, and of the various opinions that have been held regarding its systematic position, has been summarized by Professor Bower¹ in his recent paper on the Leptosporangiate Ferns, so that by way of recapitulation it is only necessary to mention the few references to the anatomy that have been found in the literature. The first of these is made by Mettenius² in 1865, and is only a casual reference to the anatomy of the stem of *Loxsoma* as differing from that of the rest of the Hymenophyllaceae (with which he includes it) on account of its 'closed vascular bundle tube.' He also notes that the root is diarch, and mentions a few facts relating to the venation. Russow³,

¹ The Morphology of Spore-producing Members; iv. The Leptosporangiate Ferns; Phil. Trans., vol. cxcii, p. 47, 1899.

² Über die Hymenophyllaceae, Abhandl. d. K. Sächs. Gesellsch. d. Wiss., Bd. vii, pp. 415 and 418.

³ Vergl. Untersuch. d. Leitbündel-Krypt., Mém. de l'Acad. Imp. de St. Pétersbourg, sér. vii, tome xix, p. 80, 1871.

later on, does no more than quote Mettenius, and then compare the structure of the vascular system to that of *Marsilea*. No mention is made at all of the anatomy of *Loxsoma* by Prantl in his paper on the *Hymenophyllaceae*, and De Bary (l. c. p. 284) only refers again to Mettenius, at the same time regretting the lack of information concerning the nature of the foliar gaps in the tubular bundle. Finally, Giesenhagen¹ confuses the correct opinion hitherto held regarding the vascular system, by speaking of it as a central collateral or concentric bundle; he also refers incorrectly to the structure of the cortex.

As regards the external morphology little additional information can be given. The rhizome is cylindrical and fairly stout (5-6 mm.). It creeps upon the surface of the soil, bearing erect leaves some two feet high, at intervals of about an inch. The leaves are situated on the upper surface of the stem, apparently in a single row, but alternating slightly on either side of the median line. They arise in acropetal succession. The stem is said to branch, but my specimens did not furnish an example. There are no localized points of departure for the roots, which arise irregularly upon the under surface and sides of the rhizome, both at the nodes, and also along the internodes. The leaves are highly dissected, and perfectly smooth and glabrous. On the stem and the base of the petiole (which is not articulated onto the stem) are a number of slight emergences which form the bases of awl-shaped structures more fitly described as hairs than as paleae. They are three or four cells thick at their base, but terminate in a single cell-row (5-12), the last cell of all being conically pointed. The walls are but little thickened and are coloured brown.

THE STEM.

In a transverse section taken through an internode in a fully differentiated region of the stem, the vascular system is easily distinguished by the naked eye as a thin, perfectly

¹ Die Hymenophyllaceen, Flora, 1890.

continuous ring of a white or grey colour, situated in the midst of the yellowish-brown ground-tissue at a distance from the centre of about a third of the radius (Plate III, Fig. 1). The ground-tissue is almost entirely composed of vertically elongated cells with square or bluntly pointed ends. The cell-walls are well thickened, with numerous round simple or slightly bordered pits, and are impregnated by some colouring substance to which is due the general brown appearance of the section. The cells of the epidermis, though less elongated vertically, do not differ from those of the ground-tissue in the structure of their walls or in the colour. Sclerenchymatous cells such as these compose the whole of the ground-tissue of the stem, with the exception of a few layers of parenchyma with thin, colourless, and unlignified walls, intervening between the endodermis and the sclerenchyma on either side of the solenostele (Fig. 2, *a, a'*); and again of a few similar parenchyma-cells that are found irregularly scattered in small groups and strands in the midst of the sclerenchyma itself. These small parenchymatous islets in the sclerenchyma only occur in the neighbourhood of the solenostele, becoming fewer the farther away from it on either side. In longitudinal section it is seen that the cells of which they are composed are much shorter than those of the thick-walled ground-tissue, that they have rounded outlines forming conspicuous intercellular spaces, and that they are arranged in vertical rows of various lengths, which appear to arise, in the apical region, by rapid and long-continued division in certain cells, while the rest grow on without further division. As regards the general sclerenchyma the cells have thinner walls and wider lumens at the very centre of the stem, and in the median region of the cortical ground-tissue (that lying without the solenostelic ring) than elsewhere (Fig. 1). The occurrence of these scattered islets of parenchyma among the sclerotic general ground-tissue of the stem is an interesting peculiarity, and I believe one of very rare occurrence among the Ferns, the only other instances which I have hitherto come across being in the stems of *Dicksonia apiifolia*, Hk.,

and *D. cicutaria*, Sw., two plants which will be later on compared with *Loxsoma* on other grounds also. The rows or strands of thin-walled cells, filled with a brown resinous tannin-containing substance, which are stated by Russow¹ to be scattered among the starch-bearing sclerotic cells of the pith and inner portion of the cortex in the rhizome of certain Marsileas (*M. salvatrix*, *M. elata*), may perhaps be regarded as somewhat similar structures.

In *Loxsoma* the distinction between these islets and the rest of the ground-tissue is well maintained in regions near the apex, even before the cells of the latter have thickened their walls; because, while the cells of the general ground-tissue are densely filled with a very finely granular almost homogeneous substance, those of the islets have scanty and coarsely granular contents. In the older parts of the rhizome small roundish or oval starch grains appear in the thick-walled cells, but not in the thin-walled cells that form the islets, although this may not hold true for all seasons of the year.

Intercellular spaces are present both in the central and the inner cortical ground-tissue, especially between the cells with thin walls. The external surfaces of the cell-walls, where they do not border upon each other, are beset with numerous delicate little rods which project into the intercellular spaces. They usually end freely in the cavity, and then they are often slightly swollen and curved at the tip; sometimes they reach right across the intercellular space and are attached to the cell-wall opposite; more rarely they anastomose with one another, or appear to branch. They are composed of some perfectly homogeneous, highly refractive substance, usually colourless, but sometimes in the older parts of the stem faint yellow. They are certainly not composed of ordinary cellulose because they are not turned blue when treated with iodine and sulphuric acid, but swell slightly and become faint yellow: treated with concentrated sulphuric acid they are dissolved, but more slowly than the rest of the cell-wall. Intercellular rodlets of this kind were first discovered by

¹ Loc. cit., p. 10.

Lueresen¹ in *Kaulfussia cicutifolia* and other *Marattiaceae*, and they have since been described in the *Ophioglossaceae*, and in a number of *Cyatheaceae* and *Polypodiaceae*².

The annular stele is limited on either side by a very distinct endodermis, consisting of vertically elongated square-ended cells, easily distinguished from the rest of the ground-tissue by reason of their very dense grey-coloured contents (Figs. 2 and 3, *e*, *e'*). Some of the cells of the endodermis differ in appearance from the rest, owing to the more coarsely granular nature of their contents, which are also impregnated with tannin. No undulations were observed on the radial longitudinal walls, but they are more or less cutinized, especially at the angles. The pericycle forms a continuous zone within the endodermis on either side of the solenostele; on the outer side, it consists of three to four layers of slightly elongated comparatively wide-lumened cells with finely pitted walls, the terminal walls being transverse or slightly oblique; on the inner side of the solenostele it is less developed, two or three layers only being present. In sections near the apex, the cells of the pericycle are seen to be superposed upon each other and upon those of the endodermis with considerable regularity, and, allowance being made for displacement due to growth and subsequent division, there is but little doubt that they all originated from the subdivision of a single cell-layer of the ground-tissue, and that therefore the pericycle is not a true but a false one (cf. Fig. 3).

The phloem, again, forms a continuous zone on either side of the solenostele, and, like the pericycle, is somewhat greater in quantity on the outer side than on the inner (Figs. 2 and 3, *ph* and *ph'*). It consists of sieve-tubes and phloem-parenchyma. The sieve-tubes have their pores aggregated into definite areas, the sieve-plates, which are present on both the longitudinal and transverse walls; further, the sieve-plates, as in the *Cyatheaceae*, are so numerous and occupy so much space that the thicker portions of the wall separating them

¹ Über den Bau und die Entwickel. der Gefässkrypt., Bot. Zeit., 1873, p. 640.

² Cf. De Bary, Comp. Anat. (Engl. ed.), p. 118.

from each other are reduced to such an extent that they form a coarse network only, the meshes of which are occupied by the sieve-plates themselves. Those elements of the phloem furthest away from the xylem on either side of the solenostele are very narrow, angular and flattened, with thick and glistening walls; in older regions almost disorganized. They constitute the protophloem, which is very clearly to be distinguished on either side of the solenostele. The central portion of the stelic ring is occupied by the xylem, which consists of scalariform tracheides with a few cells of intervening parenchyma; a layer of the latter also surrounds the xylem-strand, separating it from the phloem on both sides. The tracheides of the xylem increase in size from without towards the centre of the rhizome (Fig. 2), the smallest elements being on the outside. In sections near the apex, where the xylem is not yet fully developed, it may be seen that the smallest tracheides at the external periphery of the strand are the first to be differentiated (Fig. 3, *prx*), and it is especially to be observed that they are not collected into definite localized groups, but are fairly evenly distributed all round the external periphery. These first-formed elements are all scalariform tracheides; even after most careful research I have failed to discover any annular, reticulate, or spiral elements whatever in the stem, except on the upper surface just at the point of insertion of a leaf-trace, and these clearly are to be considered in relation to the latter. It is true that these first-formed elements of the xylem differ from typical protoxylem both in distribution and structure, and that they are only to be distinguished from the rest of the strand by their smaller size and early development, yet I think it is permissible to state that the *protoxylem proper to the stem in Loxsoma consists of narrow scalariform tracheides evenly distributed around the external periphery of the xylem-ring*. The walls of the scalariform tracheides of *Loxsoma*, in common with those of other Ferns and also of *Marsilea*¹, when treated with caustic potash or any other macerating reagent, partially dissolve, in such a manner as to

¹ Cf. Russow, Vergl. Untersuch., loc. cit.

split up along a spiral line, the spiral band thus formed being several bars of thickening in breadth (Fig. 9).

Attempts were made to obtain microtome sections of the stem-apex, but the material was not sufficiently well preserved to give good results, and the details of the apical development could not be followed. The termination of the stem is broad and flat, and from this the extreme apex arises rather abruptly as a small cone terminated by an apical cell. The solenostele was clearly delimited from the ground-tissue on both sides up to a point very near the apex, but I was unable to decide whether the lines of delimitation coincided with the first two tangential divisions in the apical segments, as stated for Ferns by Van Tieghem¹, or not. One point, however, came out with sufficient clearness regarding the origin of the so-called pericycle, which certainly arises from centripetal divisions (i. e. towards the centre of the solenostele) in a layer of cells which also gives rise to the endodermis. This holds good for both the internal and the external pericycle. In *Loxsonia*, therefore, the phloeotermis is not coincident with the endodermis, but is to be found in the innermost layer of the pericycle bordering upon the phloem.

THE ROOT.

The structure of the root is essentially that of a typical Fern. There is a slender diarch xylem-plate, the protoxylems of which abut directly on the pericycle, which is one, or in places two, layers thick. Protophloem and phloem are very distinct, and the latter is separated from the xylem by a layer of parenchyma. The endodermis is well marked, and similar to that in the stem. The cortex consists of four or five layers of cells which increase in size from the endodermis outwards; their walls are somewhat thickened and coloured brown. The cells of the suberized layer are radially elongated with especially thick walls; they usually remain as the outer limit of the root, the piliferous layer being early destroyed. The

¹ *Traité de Botanique* (2nd ed.), p. 774.

root-steles arise from the under-surface and sides of the solenostele of the rhizome, and the long axes of their diarch xylem-plates are so arranged that they are tangential to the solenostele in a transverse plane. The root-steles pass out in a very slightly oblique direction towards the apex.

THE PETIOLE.

The only points at which the perfect continuity of the closed solenostele is interrupted are the points of insertion of the leaf-traces. At each node the departure of a vascular strand to supply the leaf removes a portion of the vascular ring bodily from the stem, leaving a gap through which the parenchyma and sclerenchyma of the ground-tissue lying within the vascular ring communicate with the corresponding tissues lying without it, while at the same time the internal endodermis, pericycle, and phloem become continuous with the external, around the margins of the gap. The leaf-gap thus formed persists for some little distance (6-8 mm.) into the internode above, but gradually becomes closed up again. The manner in which the leaf-trace actually arises from the solenostele of the stem was ascertained by the comparison of a number of consecutive transverse sections taken through a node, and the conclusion confirmed by rough dissections. As a result, Fig. 4 was drawn as the representation of the form taken up by the vascular system of the rhizome at the nodes, and it is hoped that a reference to it will facilitate further description. It is seen in the diagram that the portion of the vascular system of the stem destined to pass off as a leaf-trace is clearly indicated for a short time previous to its departure as a protuberance on the upper side of the solenostele, and a reference to transverse sections shows that this leaf-trace portion rapidly diminishes in thickness as the point of departure is approached. The drawing is further intended to show that the leaf-trace (which has the form of a trough, or in transverse section that of a horseshoe) is seated upon the vascular tube of the stem almost in the

middle line, with its convexity directed towards the base of the stem and its concavity towards the apex.

The transverse section of the free petiole is approximately oval, and its vascular strand, which lies towards the centre, is curved into the form of a horseshoe with the concavity directed adaxially (Fig. 5). The limbs of the horseshoe are enlarged towards their extremities; it varies in degree of curvature according to the level in the petiole at which the section is taken, the limbs being more widely separated and the whole strand less curved towards the top of the petiole. Towards the base of the petiole the ground-tissue is exactly like that of the stem, being entirely sclerenchymatous, with the exception of two or three layers of parenchyma immediately surrounding the vascular strand, and a few similar elements scattered amidst the more central sclerenchyma. However, at a point higher up, the brown sclerenchyma at the periphery of the petiole becomes changed into a hypodermal zone of elongated finely-pointed fibres with thick colourless walls and slit-like pits, while all the rest of the ground-tissue gradually becomes thin-walled and parenchymatous. The brown sclerenchyma persists longest within the concavity of the horseshoe, and along its flanks. At rare intervals, the dense sclerenchyma occupying the periphery of the petiole is interrupted by short tracts of loosely packed cells visible on the outside as short grey streaks. In longitudinal section it is seen that these tracts consist of elongated sausage-shaped cells, with rounded ends whose longitudinal walls are not in close contact all along their length, but separate from each other at intervals, so that a number of intercellular spaces of different sizes appear between them. These intercellular spaces give the tissue a distinctive appearance both in longitudinal and transverse section, although in the thickness of their walls and in other respects its cells closely resemble those of the rest of the ground-tissue. The few stomata that are to be found upon the petiole are localized above these tracts of tissue. The petiolar parenchyma in a very large number of Ferns is

interrupted by similar tracts of spongy air-filled tissue; most frequently it occurs as a single continuous line on either side of the petiole; often the line is more or less interrupted, or indeed, as in *Loxsoma* itself, completely broken up, so that this tissue is represented by a number of short streaks which are not even arranged in the same straight line, although they are still aggregated towards the sides of the petiole. The very general distribution among the Filicales of this tissue, which might be regarded as representing modified traces of a mesophyll decurrent along the sides of the petiole, is a very significant phenomenon in reference to the view advanced by Professor Bower¹ concerning the leaf of the Ferns, which he regards as a rachis or *phyllopodium*, fundamentally winged along its whole length.

The xylem occupies the centre of the vascular strand of the petiole, the outline of which it accurately follows, except in the enlarged ends of the flanks of the horseshoe, where the xylem-strand is bent on itself, forming a hook which is curved inwards and encloses a small bay between itself and the rest of the xylem (Figs. 5 and 6, *hk*). The phloem surrounds the xylem completely but not evenly, being in greatest quantity on both sides of the flanks, while on the median concave region of the horseshoe it is very scanty.

The protophloem, with its flattened angular elements and swollen walls, is well marked, especially on the flanks, but it is entirely absent in the median concave region (Fig. 5, *pph*). The pericycle consists of two or three layers, except in the median region, where only one is present; on the outside of the flanks of the horseshoe its cells are considerably larger than elsewhere. In examining the petiolar meristele of *Loxsoma* attention is at once attracted to the presence of a number of sclerosed fibrous elements situated in close proximity to the periphery of the xylem, such elements being of very rare occurrence in the vascular strands of Ferns. These

¹ Comp. Morph. of the leaf of the Vasc. Crypt.; Phil. Trans., vol. clxxv, 1884, p. 606.

sclerotic fibres are found in greatest quantity at the inner side of the phloem along the flanks of the horseshoe, and especially in the bays formed by the hooked ends of the xylem. One or two layers of the same accompany the concave internal surface of the xylem-strand, entirely replacing the phloem in the median region, and a few also occur on the external convex surface, forming an interrupted layer between the xylem and the phloem (Figs. 5 and 6, *sc. t.*). Fibrous elements, almost identical in position and appearance with those of *Loxsoma*, have been carefully described in the petiole of *Aneimia Phyllitidis* by Prantl¹, who regards them as belonging to the phloem. Poirault² goes further; for he considers some very similar elements he observed in the petioles of certain *Gleichenias* actually to represent sieve-tubes which have undergone sclerosis. Evidence is not wanting which would lead to a similar conclusion with respect to the fibrous elements in *Loxsoma*. In the first place, the position that they occupy in the meristele relative to the other tissues is precisely that of sieve-tubes, and, wherever they occur in mass, thin-walled parenchyma-cells are scattered amongst them, which may be regarded as representing the phloem-parenchyma (Fig. 6, *pa*). Again, towards the base of the petiole they gradually decrease in number (those in the bays of the hooks persisting longest), until at the point where the leaf-trace enters the tissue of the stem they disappear altogether, their place being progressively taken up by undoubted sieve-tubes. They are usually separated from the xylem by a layer of thin-walled parenchyma, but occasionally are found in direct contact with a tracheide (Fig. 6, at +). This, however, does not affect our comparing them with sieve-tubes, because the latter are also sometimes found in the same position, both in *Loxsoma* and in other Ferns.

Moreover, as seen in *Loxsoma*, these fibrous elements closely

¹ Untersuch. z. Morph. d. Gefässkrypt., Heft ii, Schizaeaceen, p. 28 (Leipzig, 1881).

² Recherches Anat. sur les Crypt. Vasc., Ann. Sc. Nat., sér. vii, tome xviii, p. 190, and Fig. 18, 1893.

resemble sieve-tubes in their structural details, being very elongated and tubular, and arranged more or less in series with very oblique terminal walls. The walls are greatly thickened and more or less lignified, but even when strongly so they are easily distinguished from the tracheides by the rounded inner contour of their lumen (Fig. 6). Two fairly distinct layers of thickening have been deposited upon the middle lamella. A number of round or elliptical simple pits are scattered over the walls (Fig. 10), and in immature stages (especially on the terminal walls) they are so numerous and of such a size that they give the impression of a coarse reticulation, reminding one strongly of the sieve-tubes themselves. They never appear to be quite empty, but have much denser contents than the sieve-tubes, and nuclei are frequently to be observed in them. It would seem, therefore, that both their distribution and their structure would most readily be explained if they were regarded as elements of the phloem originally designed for sieve-tubes, but which, in the course of their development, have thickened and lignified their walls to such an extent that the areas which should have become sieve-plates have become simple pits, and the whole element more closely resembles a fibre than a sieve-tube. It is not implied, of course, that they were all at one time actually functional sieve-tubes. Further support for this point of view may be drawn from the fact that a number of elongated elements with thick two-layered but unlignified walls, occupying precisely the same position in the petiolar meristele as do the fibrous elements in *Loxsoma*, may be found in many *Microlepias* (*Davallia platyphylla*), and *Dennstaedtia* (*Dicksonia punctiloba*), where they can only be interpreted as representing sieve-tubes.

Although the above explanation seems highly probable for the particular plants in question, yet it certainly is not safe to apply it to all the other cases in which fibrous elements have been found in the petiolar meristeles of Ferns. However, it seems to account satisfactorily for the cases of *Lygodium*, *Schizaea*, and *Aneimia* mentioned by Prantl (l.c.), and also

for that of *Matonia pectinata*¹. On the other hand, in *Trichomanes Prieurii*, where these fibres were first discovered by Mettenius (l.c.), all the sclerotic elements can hardly be regarded as sclerosed sieve-tubes, because, as I have seen, although some are elongated and pointed, resembling those in *Loxsonia*, there are also others which are relatively short and square-ended; here, perhaps, both constituents of the phloem, sieve-tubes, and parenchyma are implicated in the sclerosis. Finally, a very puzzling case is described by Thomae² in the petiole of *Adiantum trapeziforme*, var. *pentadactylon*, where the fibrous elements are found not only at the margin of the xylem-strand, but also intermingled among the tracheides themselves. The most obvious suggestion seems to be that the xylem-parenchyma has become sclerotic and fibrous.

The xylem, apart from its first-formed elements, consists of scalariform tracheides, which upon treatment with caustic potash behave in the same way as those in the stem. The largest tracheides are found on the flanks of the xylem-strand, and the smallest in the neighbourhood of the protoxylem-groups. The protoxylem consists of narrow elements exhibiting beautiful spiral and angular thickenings, and is situated on the internal adaxial surface of the xylem-strand, towards the concavity of the horseshoe; it may therefore be regarded as endarch (Figs. 6 and 8, *prx*). The number of groups varies according to the petiole, and in the same petiole according to the level at which the section is taken, becoming fewer towards the top of the rachis. Generally speaking, there are two or three groups in the bay of each hook, and from two to six along the dorsal-curved portion of the strand. These endarch protoxylems, although perfectly clear and distinct in all parts of the free petiole, gradually become less definite when the petiolar meristele enters the tissues of the stem. Shortly after the leaf-trace has become completely

¹ Seward, Struct. and Affin. of *Matonia pectinata*, Phil. Trans., B., vol. cxcii, 1899, p. 183.

² Blattstiele der Farne, Prings. Jahrb., Bd. xvii, 1886, p. 129.

continuous with the upper surface of the solenostele, they disappear altogether without at any time coming into relation with the scalariform protoxylem-elements that line the external border of the lower part of the solenostele.

In the leaf-trace, therefore, the xylem is developed centrifugally from definite and typical protoxylem-groups. On the other hand, it has been shown that in the stem the development of the xylem is not related to the leaf-trace protoxylems decurrent in the stem, nor to any other definitely localized groups; neither are there any elements present in the stem which exhibit the characteristics typical for protoxylem. Since the whole mass of xylem in the stem cannot well be regarded as metaxylem (primary xylem other than protoxylem), I find it necessary to accept the alternative, and to recognize in *Loxsoma* two kinds of protoxylem differing in structure and in disposition; one peculiar to the stem, and the other to the leaf. Particular attention has been drawn to this distinction because it may prove a factor of considerable importance in the estimation of the influence exerted by the anatomy of the leaf upon that of the stem, in cases where the former is the predominant member of the plant, a subject which does not seem to have attracted the attention it deserves.

The xylem is almost entirely free from parenchyma scattered among its tracheides, but at the same time the whole strand is surrounded by a well-defined layer of cells exactly similar to those of xylem-parenchyma. However, the cells of this xylem-sheath (Fig. 6, *x. sh*) are not all alike, for those in the neighbourhood of the protoxylem-groups undergo such profound and peculiar modifications that they have been considered to be a new kind of tissue. The cells at these points become greatly enlarged by growing out into the space left vacant by the disintegration of the protoxylem-elements; their walls, which are considerably thickened and sometimes even lignified, are thrown into a number of deep irregular folds or pleats, especially on the side facing the protoxylem (Fig. 11, *cp*). They are covered by a number of large simple pits, often to such an extent that they appear to be coarsely

reticulated. They contain a fair amount of protoplasm and a conspicuous nucleus. Cells somewhat similar to these were first mentioned by Dippel¹ in *Osmunda* and *Cyathea*; he regards them as reservoirs for bye-products such as resin, tannin, &c., but in *Loxsoma* no trace of these substances is to be detected. The tissue described by Russow² in *Marsilea* under the name of cavity-parenchyma (Lückenparenchym), and again by Thomae (l.c.) in *Angiopteris* and *Cyathea* under the title of 'Stumpffzellstränge,' is almost identical with that in *Loxsoma*. Thomae also states that the papillae may become cut off by walls from their mother-cells, on which account he compares this to a case of thylosis. Other examples have recently been given by Seward in *Matonia pectinata* (l.c.), and by Farmer³ in *Helminthostachys*; indeed it has been mentioned by various observers as occurring in all the main families of Ferns, although the exact limits of its distribution are yet to be determined.

Towards the top of the petiole the vascular strand, although still resembling a horseshoe, is markedly different from the form already described. Taking it, for instance, at a point just below the insertion of the lowest branches of the leaf (Fig. 7, *b*), it is seen that the flanks of the horseshoe are much wider apart from each other, and that its concavity is more shallow; in fact, the whole meristele appears to be somewhat flattened out. The departure of the vascular strands for the successive branches, which are nipped off from the ends of the arms of the meristele, does not materially alter its form, although causing it to decrease considerably in size. At the same time, however, the hooks at the ends of the xylem-curve gradually disappear, and the protoxylem-groups become fewer in number until three only are left; one at the end of each arm and one in the median region of the curved xylem (Fig. 7, *c*). From now onwards, this median protoxylem sinks further and further away from the

¹ Das Mikroskop, Theil ii, p. 201, Fig. 100, 1867.

² Loc. cit., pp. 6 and 101, and Taf. ii, Fig. 13.

³ Ann. Bot., vol. xii, no. 51, p. 439, 1899.

adaxial surface of the meristele, until it comes to occupy the bottom of a deep bay between the arms of the xylem, which for some time have been gradually approaching each other until they are now almost in contact. At length, therefore, owing to its diminished size, and to the flattening-out of its formerly concave side, the form of the meristele has changed by degrees from that of a horseshoe to that of an equilateral triangle with rounded angles and convex sides, the adaxial side being slightly indented at the centre (Fig. 7, *d*, and Fig. 8). Nevertheless, an indication of the form it originally possessed is still to be perceived in the curve that is retained by the xylem-strand, even when the outline of the meristele itself has become elliptic. While these changes are progressing the cavity-parenchyma becomes less and less distinct, disappearing altogether before the meristele becomes elliptic. The sclerosed sieve-tubes, on the other hand, are still to be observed in considerable quantity (Fig. 8), and continue to persist even in the small veins of the lamina itself. The vascular strands of the lateral branches of the leaf pass through exactly the same changes until the ultimate ramifications of the veins in the foliar segments are reached: there they exhibit a perfectly collateral structure, the phloem being confined to the abaxial side of the xylem, at the adaxial extremity of which is situated a single protoxylem-group (Fig. 7, *e*). Although the mesophyll of the lamina is of considerable thickness, no palisade arrangement of the cells is to be found in it. On the contrary, they are all arranged in a loose spongy manner with numerous intercellular spaces, which, however, are particularly large towards the under surface of the leaf. The cell-walls limiting these intercellular spaces are closely beset with those intercellular rodlets already described in the stem. The veins in the lamina are connected to both the upper and the lower surfaces by stout strands of sclerenchyma after the manner of girders, even the epidermal cells overlying these veins are more or less thickened and elongated in the direction of their course. The cells of the general epidermis

are very irregular in shape, with beautifully undulated outlines. There are numerous stomata on the under-surface of the leaf, which are overtopped by the neighbouring epidermal cells in such a manner that the outer walls of the guard-cells are at the level of the inner walls of the cells of the general epidermis (Fig. 12). The external longitudinal ridges of the guard-cells are well developed and highly cutinized; but the internal are absent, although the corresponding region of the wall becomes cutinized. I was unable to ascertain the way in which the stomata were developed owing to absence of a sufficiently young leaf.

GENERAL CONSIDERATIONS.

In seeking to determine relationships between *Loxsonia* and the other Ferns, using the anatomy as a guide, I venture to regard the solenostely of the stem and the peculiar horse-shoe shape of the petiolar meristele as characters of primary importance, and of considerable reliability. Judging from this point of view, it would appear that the nearest allies to *Loxsonia* are to be sought for among those more primitive *Cyatheaceae* and *Polypodiaceae* that are included, together with *Loxsonia* itself, in Bower's proposed sub-order *Gradatae*¹. More especially is it related to certain species of *Dennstaedtia* and *Microlepia* (given by Hooker as sections of the genera *Dicksonia* and *Davallia* respectively). It is difficult to be more precise upon this point at present, because neither of these two sections, as at present constituted, are altogether homogeneous in their anatomical aspect; at least, so far as I have been hitherto able to ascertain. Nevertheless, the majority possess a typically solenostelic vascular system, and a hippocrepiform petiolar meristele, just as in *Loxsonia*. As regards minor detail, in most of the species of both sections the protoxylems in the petiole are bordered by cavity-parenchyma, and elements resembling the sclerosed sieve-tubes

¹ The Morphology of Spore-producing Members; iv. The Leptosporangiate Ferns (loc. cit.).

are also present. In many cases also, the cells bounding the intercellular spaces of the mesophyll are beset with the small rod-like structures described in *Loxsoma*. If it is necessary to select any particular species for still closer comparison, *Dicksonia apiifolia*, Hk. (*Dennstaedtia*), and *D. cicutaria*, Sw. may be referred to as perhaps most suitable for the purpose. In the first place, these are the only two Ferns that I am as yet aware of, besides *Loxsoma*, in which the protoxylem-elements of the stem are all scalariform, and not aggregated into definite groups, but evenly distributed around its external periphery. Further, the small islets of parenchyma scattered amongst the sclerenchymatous ground-tissue, that form so characteristic a feature of the stem in *Loxsoma*, are also to be found in these two *Dicksonias*, with the same little rodlets projecting into their intercellular spaces. Cavity-parenchyma is present in the hippocrepiform petiolar meristele of *Dicksonia apiifolia*, and the sieve-tubes situated in the bays of the hooks of the xylem-strands have especially thick walls although they remain unligified. Whether the petiole of *Dicksonia cicutaria* presents the same structure I did not ascertain, because my material was accidentally lost, and I have not been able to replace it.

The affinity thus brought to light between *Loxsoma* and the *Dennstaedtiinae* is in no wise discountenanced by comparisons which may be drawn from the morphology of the sorus and sporangia. For Professor Bower (l. c.) has shown that in this respect also a number of important characters are held in common by them both; such as the marginal position of the receptacle, the position and form of the indusium, the basipetal succession of the sporangia, the obliquity of the annulus, and the relatively low output of spores (64). It must be borne in mind, however, that the consideration of the sorus and the sporangium also indicates affinities as close, or even closer, to the *Gleicheniaceae* and *Hymenophyllaceae*. It is true that suggestive parallels may be drawn between the anatomy of *Loxsoma* and that of certain members of these families, but it seems to me that

they are of too speculative a nature to compete with the more pronounced relationship between it and the *Dennstaedtiinae*.

To begin with the *Gleicheniaceae*. No case of simple solenostely is known in this family, the nearest approach among allied groups being the highly complex and remarkable vascular arrangement recently described by Seward (l. c.) in *Matonia pectinata*, R. Br., the explanation of which I am inclined to seek in the profound modification of some more simple solenostelic ancestor. It is interesting to discover that the form of the petiolar meristele of *Matonia* is a slight modification of the horseshoe, as indeed is also the case in *Gleichenia* itself, where the arms of the horseshoe are curved inwards to such an extent that they meet together in the median line, thus enclosing a small mass of sclerenchymatous ground tissue in the centre of the horseshoe. However, at one level or another in the petiole of *Gleichenia dichotoma*, generally near the base, I have found that the arms of the horseshoe separate from one another so that the enclosed ground-tissue becomes continuous with that outside the meristele; thus the hippocrepiform nature of the meristele becomes quite clear. It should be mentioned that both sclerosed sieve-tubes and cavity-parenchyma are to be found in *G. dichotoma*.

As regards the *Hymenophyllaceae*, the universal occurrence in the stem of a solid central cylinder clearly places them anatomically much farther away from *Loxsonia* than are the Cyatheaceae, and this estrangement is deepened by the plentiful display of stomata and intercellular spaces in the lamina of the leaf of *Loxsonia*, which are never to be found in the *Hymenophyllaceae*, not even when the lamina is several layers thick, as in *Trichomanes reniforme*. For all that, there are several points that suggest a certain relationship, although it may be a somewhat distant one. It is especially in the vascular strand of the petiole that these points are to be sought; for, although the outline of the vascular strand as a whole is, in most of the stouter species, oval or roughly triangular, yet I believe it possible to

recognize indications of a horseshoe design in the more or less curved form of the xylem-strand. This possibility had already suggested itself to me before I had the opportunity of reading the interesting paper recently published by Boodle upon the anatomy of this order¹. He there describes a type of petiole, previously unknown to me, which goes far to bridge over the gap that existed between the petiolar strands of the *Hymenophyllaceae* and the hippocrepiform meristele of the petiole of *Loxsoma*. This type is to be found in *Trichomanes apiifolium*, Presl, where not only the xylem-strand, but also the outline of the meristele itself, has the form of a horseshoe or arch, and further, the ends of the arms of the xylem are prolonged into incurved 'hooks' precisely as in *Loxsoma*. It also appears that there is a protoxylem-group in the bay of each hook, and probably one in the middle of the curved region. *Trichomanes Prieurii*, Kz., is also a most instructive type; for here, although the xylem-strand exhibits the same form as in *T. apiifolium*, sometimes with two protoxylem-groups on the curved region, the outline of the meristele no longer follows that of the xylem, but is approximately reniform. This state of affairs will permit of a fairly close comparison with the meristele in *Loxsoma* at a point near the top of the rachis, where it becomes diminished in size and simplified in form (cf. Fig. 7, c), and especially so since this particular *Trichomanes* also presents cavity-parenchyma and fibrous elements, some of which may represent sclerosed sieve-tubes. Granted these two examples, the petiolar strands of many other species fall into line as a series of progressive simplifications of the horseshoe design in accordance with the diminution in their size. Thus, in those of the type of *Trichomanes scandens*, L., the hooks at the ends of the arms of the xylem have practically disappeared, leaving a Λ or crescent-shaped strand with a protoxylem at the end of each arm and one median dorsal. The outline of the meristele itself is now circular. By the disappearance of the median protoxylem a still simpler type is produced,

¹ Ann. Bot., vol. xiv, p. 455, 1900.

such as in the stouter Hymenophyllums, where the xylem-strand has the form of a crescent with a protoxylem-group at either end. Judging from a diagram by Prantl, *T. Bancroftii* is somewhat after the same type, with the meristele flattened out, and the hooks of the xylem-band persistent, but closely adpressed to the curved position. According to the same authority the xylem-strand of the petiolar meristele of *T. tanaicum* also has the form of a crescent with two protoxylems, one at either end, but with the phloem entirely confined to the abaxial surface of the xylem, the adaxial concavity being occupied by a group of fibrous elements.

There are a number of points in the anatomy of the *Schizaeaceae* again that may be used for comparison with *Loxsonia*, but none are of sufficient weight to constitute a close relationship. For instance, there is at least one species of *Aneimia* (*A. Mexicana*), in which a closed vascular ring is found in the stem. Through the kindness of Mr. Boodle, who is about to publish an account of the anatomy of this Order, I was enabled to examine a section of this plant, but there is little to be found in the exiguous vascular strand of the *Aneimia* that may be compared with the solenostele of *Loxsonia* save the bare form alone. It appeared to me that the vascular tissue of the *Aneimia* had undergone a great amount of reduction, in which opinion, I believe, Mr. Boodle also concurs. A much more striking analogy between *Loxsonia* and the *Schizaeaceae* is to be found in the exarch evenly distributed protoxylem-elements of the stem of *Schizaea* and *Lygodium*; further, in *Schizaea*, and possibly also in *Lygodium*, the elements appear to be all scalariform. The petiole of *Aneimia* (*A. Phyllitidis*) exhibits again an almost typical example of a hippocrepiform meristele with cavity-parenchyma and sclerosed sieve-tubes. Too little is known about the detailed anatomy of the *Schizaeaceae* as a whole to drive the comparison further.

SUMMARY.

To sum up shortly, the principal features of the anatomy of *Loxsoma* and the conclusions resulting from their consideration may be stated as follows:—

1. The stem is typically solenostelic.
2. A single vascular strand is given off to each petiole having the form of a horseshoe, the xylem being of the same form, and hooked at the ends.
3. In the stem the protoxylem-elements, which are all scalariform, are not localized into definite groups, but are distributed around the external periphery of the solenostele.
4. In the petiole the protoxylem-elements, which are spiral and annular, are collected into definite *endarch* groups, but they are not continued down into the solenostele of the stem.
5. Parenchymatous islets are found in the sclerenchyma of the stem, and intercellular rodlets in the tissues of both stem and leaf.
6. Sclerosed fibres and cavity-parenchyma are present in the vascular strand of the petiole.
7. By its anatomy *Loxsoma* is more nearly related to the *Dennstaedtiinae* and the solenostelic *Polypodiaceae* than to any other family of Ferns, although affinities of a more remote nature to the *Gleicheniaceae*, *Schizeaceae*, and *Hymenophyllaceae* are also indicated.

CONCLUSION.

In reviewing the anatomy of a number of Fern-petioles I have been greatly impressed by the fact that the plan of the vascular arrangement in the petiole is almost invariably referable to a single universal design, that which I have described as resembling a horseshoe. So much, indeed, is this the case that I have been led to regard it, with some notable exceptions (*Lygodium*, *Schizaea*, *Marattiaceae*, &c.), as fundamentally typical for the Filicales. In many cases, of course, the

original design has become more or less masked by the splitting up of the horseshoe into a number of separate portions, which portions may subsequently fuse or anastomose with each other in different ways; yet it is rarely very difficult to trace back such arrangements to the typical scheme. I lay some stress upon this point because, in the Ferns, where such a continuity of design is apparent, I am inclined to regard the modifications in the structure of the leaf-trace as being in a manner complementary to those of the stelic system of the stem, and taken together as presenting material which deserves very careful consideration in discussing the relationships of the plants themselves. I believe that the simplest form of this general design (an undivided horseshoe such as in *Loxsoma*) may be regarded with tolerable safety as being relatively primitive, and that therefore its occurrence together with a primitive stem-anatomy may be used as additional evidence of much weight regarding the relative position of the plant as a whole.

Finally, I cannot refrain from referring to one other point which the study of *Loxsoma* forcibly accentuates, and that is, the general similarity that exists between the anatomy of the solenostelic Ferns and that of *Marsilea*. Allowing for a certain amount of simplification due to a water-habitat, *Marsilea* differs from them in no essential feature, neither in the structure of the solenostele, the departure of the leaf-trace, nor in the form and structure of the latter. As regards the leaf-trace, it is almost a facsimile of the petiolar meristele of *Loxsoma* when reduced in size far up the rachis (Fig. 10, *d*). Cavity-parenchyma is present, and the sieve-tubes in the deep bay opposite the median protoxylem, although they are not exactly sclerosed, are exceptionally thick-walled. A similar parallelism is found in other details, such as the splitting of the scalariform tracheides along a spiral line when macerated, &c. The leaf-trace protoxylems do not appear to be prolonged down into the stem. In the stems of species with a tolerably stout xylem-ring there do not seem to be any localized protoxylems, although the smaller

elements are found towards the external periphery. Russow also comments upon the absence, or at any rate the extreme rarity of spiral or annular elements in the stem. These features are quoted with the intention of pointing out the general anatomical similarity of *Marsilea* to a simple plan of Fern-anatomy, but without drawing the comparison definitely with any individual genus or order.

I take this opportunity to express my gratitude to Professor Bower for the interest he has taken in my work, and for the valuable advice and assistance he has given me in the construction of this paper.

I have also to thank Dr. W. H. Lang for useful advice upon many points.

EXPLANATION OF THE FIGURES IN PLATE III.

Illustrating Mr. Gwynne-Vaughan's paper on *Loxsoma*.

Figs. 1, 2, 3, 6, 8, and 9, are from photographs; a more or less under-exposed print was taken, and this was accentuated with a pencil; Figs. 5, 10, 11, and 12, are camera lucida diagrams; Figs. 4 and 7 are diagrams. The following lettering is used throughout: *e*, external endodermis; *e'*, internal endodermis; *P*, external pericycle; *P'*, internal pericycle; *ph*, external phloem; *ph'*, internal phloem; *pph*, external protophloem; *pph'*, internal protophloem; *x*, xylem; *prx*, protoxylem.

Fig. 1. Portion of a transverse section of an internode of the rhizome: *ss*, solenostele; at the zones marked (x) the ground-tissue is more sclerotic than elsewhere. The solenostele is preparing to give off a leaf-trace on the left. $\times 12$.

Fig. 2. Portion of a transverse section of the solenostele: *a*, *a'*, zones of parenchyma intervening between the two endodermal layers (*e*, *e'*) and the sclerenchyma of the ground-tissue. $\times 150$.

Fig. 3. Portion of a transverse section of the solenostele towards the apical region of the stem. The xylem is not yet completely differentiated, although the protoxylem elements (*prx*) are already well thickened and lignified: *m*, unthickened elements of the metaxylem. $\times 100$.

Fig. 4. Diagram showing the form of the vascular system at the nodes of the rhizome: *ss*, solenostele; *lt*, departing leaf-trace; *lg*, leaf-gap. The arrow points towards the apex of the rhizome.

Fig. 5. Transverse section of the petiolar meristele at a point below the middle of the petiole; slightly diagrammatic: *hk*, the hooked ends of the xylem-strand;

sc. t., sclerosed sieve-tubes; *lp*, cavity-parenchyma opposite each of the protoxylem-groups. $\times 50$.

Fig. 6. Left-hand corner of Fig. 6 more highly magnified: *hk*, hooked ends of the xylem-strand; *sc. t.*, sclerosed sieve-tubes; *lp*, cavity-parenchyma; *pa*, parenchyma-cells among the sclerosed sieve-tubes; *x. sh.*, xylem-sheath. At (\times) the sclerosed sieve-tubes are seen to be in immediate contact with the tracheides. $\times 260$.

Fig. 7. Diagrammatic transverse sections of the petiolar meristele in different regions of the leaf: *a*, about half-way up the petiole; *b*, just below the insertion of the lowest branch; *c*, about the middle of the branched portion; *d*, near the top of the rachis; *e*, in the principal vein of a leaf-segment. The protoxylem-groups are indicated by black dots.

Fig. 8. Transverse section of the petiolar meristele at about the stage 7, *d*, more highly magnified: *sc. t.*, sclerosed sieve-tubes; *P*, pericycle. Note the disappearance of the hooks at the ends of the xylem-strand. $\times 180$.

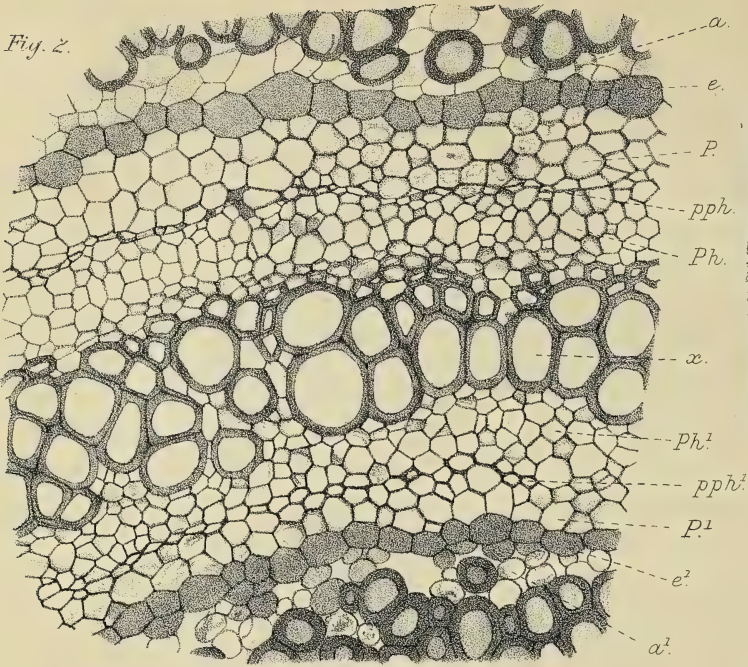
Fig. 9. A scalariform tracheide after maceration in caustic potash. It becomes unrolled into a spiral ribbon bearing several bars of thickening in its breadth. $\times 260$.

Fig. 10. Two of the fibrous elements of the petiolar meristele (sclerosed sieve-tubes) in longitudinal section. \times about 400.

Fig. 11. Cells of the cavity-parenchyma: *a*, an older, *b*, a younger stage. \times about 300.

Fig. 12. Transverse section of a stoma. Intercellular rodlets are found in the substomatal cavity. \times about 500.

Fig. 2.



ss.

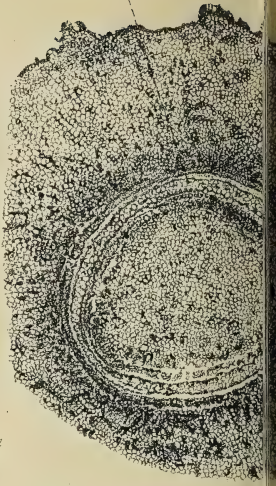


Fig. 1.



Fig. 3.

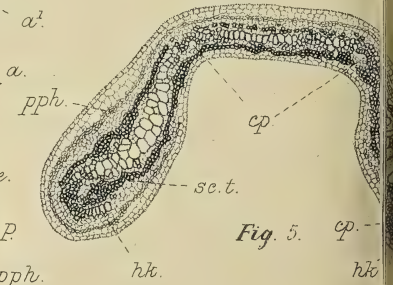


Fig. 5.

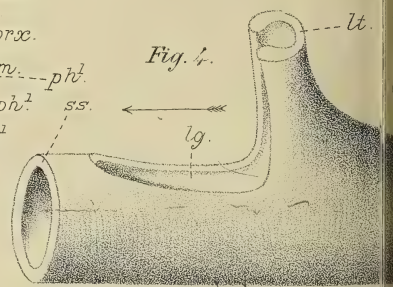


Fig. 4.

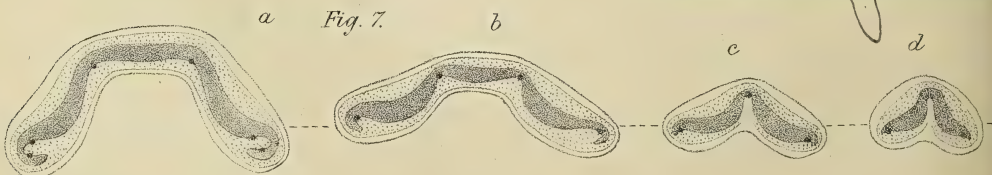


Fig. 7.

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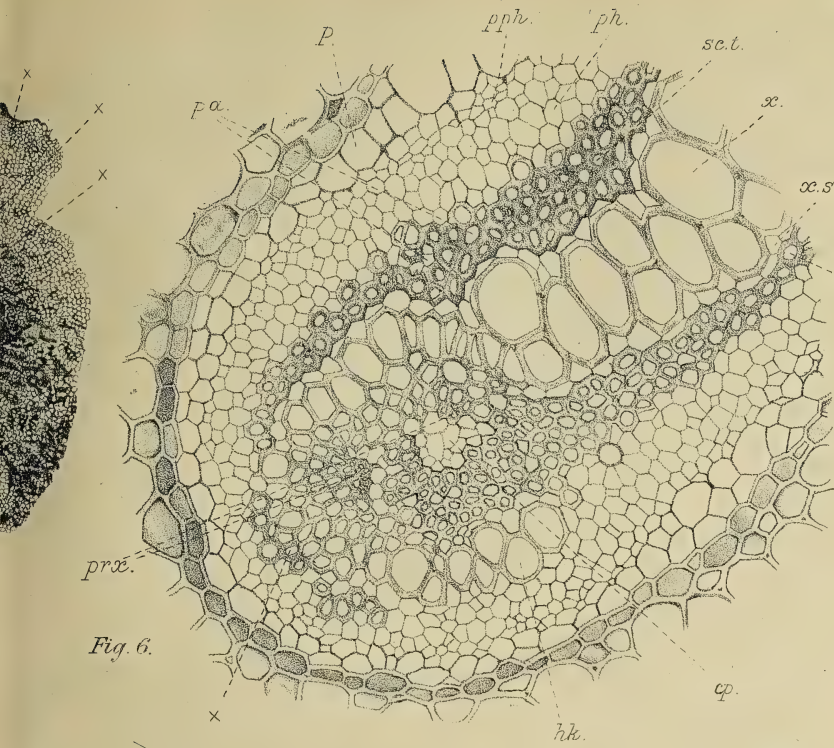


Fig. 6.



Fig. 9.

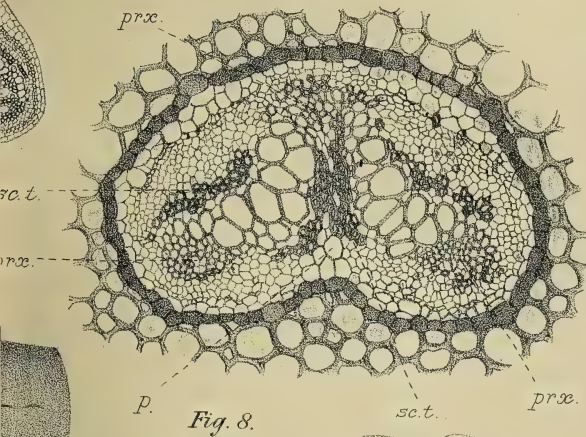


Fig. 8.

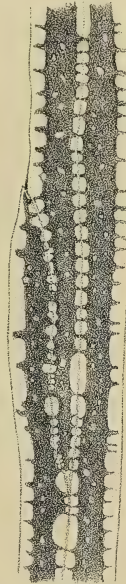


Fig. 10.

Fig. 11.

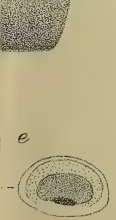
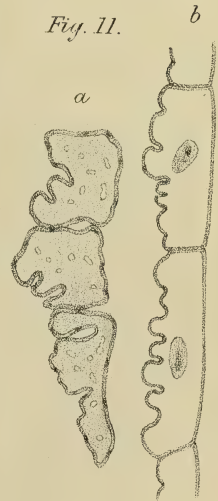
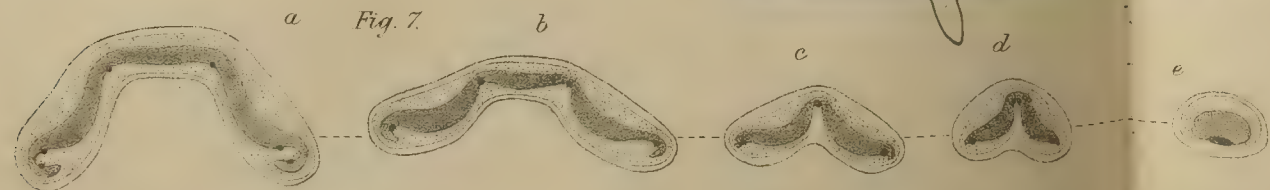
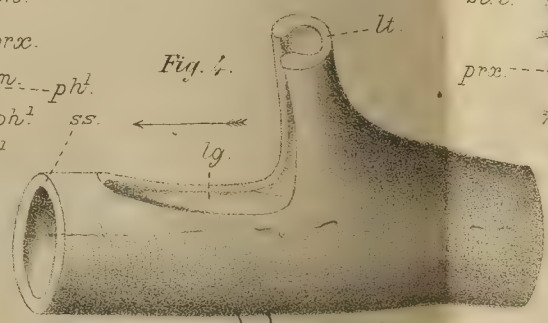
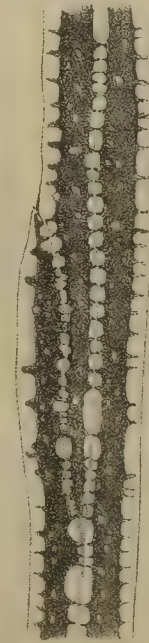
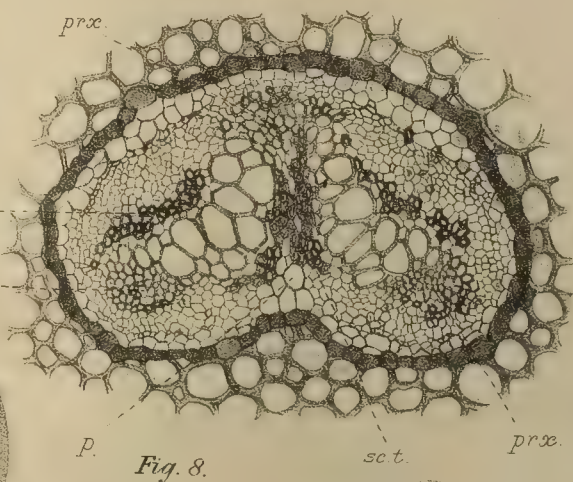
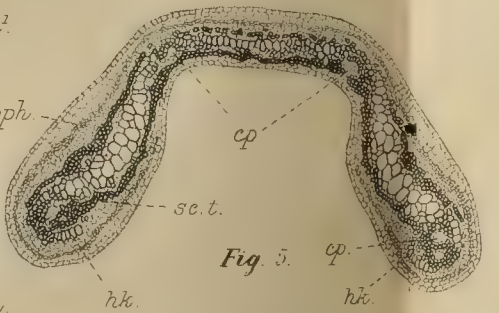
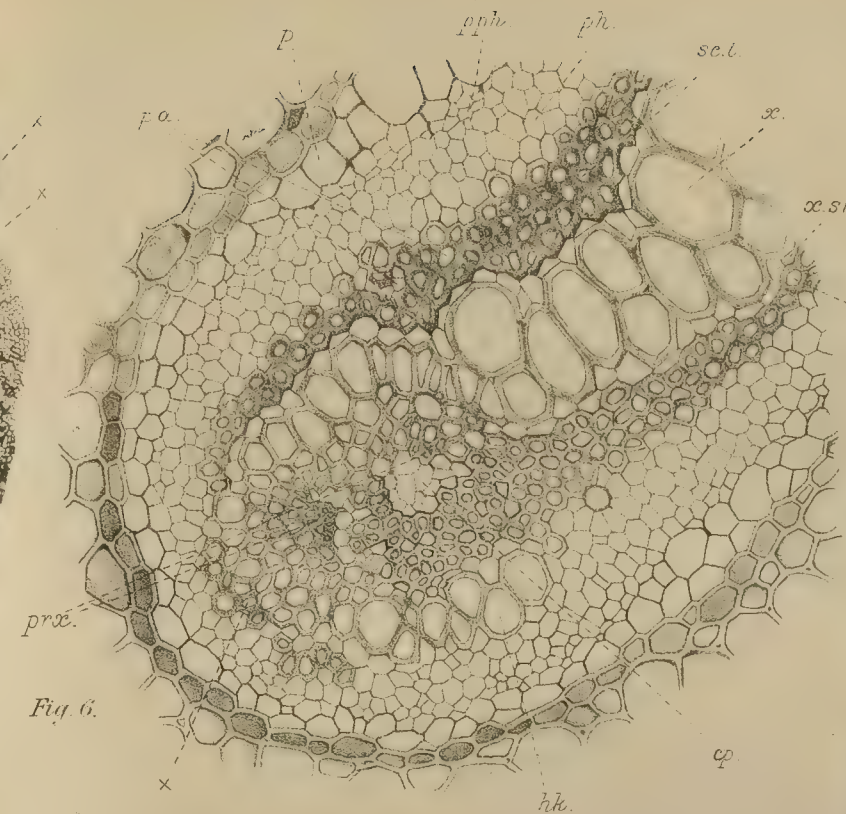
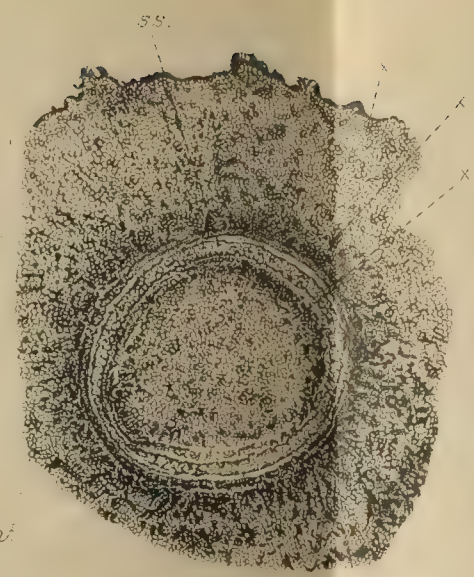
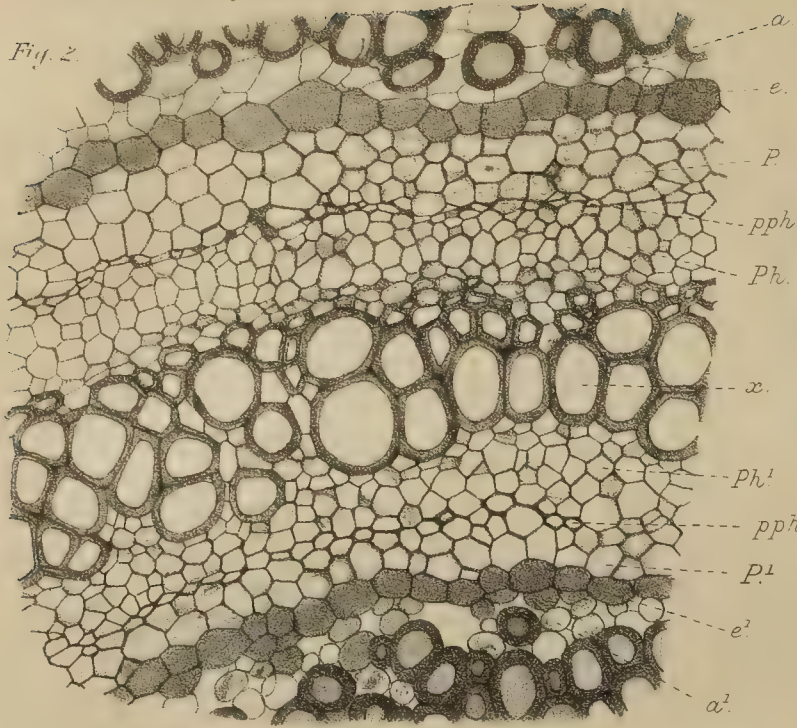


Fig. 12.



DT G-V. Photo et del.

Germination of Seeds of *Bertholletia excelsa*.

BY

WILLIAM WATSON,

Assistant Curator, Royal Botanic Gardens, Kew.

With Plates IV and V.

THE tree which yields the 'Brazil-nuts' of commerce is a native of Guiana, Venezuela, and Brazil, where it forms forests in the neighbourhood of large rivers. According to Humboldt it attains a height of a hundred to a hundred and twenty feet, the trunk two or three feet in diameter, with large open branches bearing tufts of very close foliage at their summits. The trees flower in March or April, and the fruits ripen in about two months, 'forming in less than fifty or sixty days a pericarp, the ligneous part of which is half an inch thick, and which it is difficult to cut with a sharp saw.' The fruit is spherical, about six inches in diameter, and it contains from fifteen to twenty nuts, arranged with their thin edge inwards. At the apex of the fruit there is an aperture half an inch in diameter, which is closed with a plug formed by the hardened calyx. Humboldt says this plug 'seldom opens of itself. Many seeds from the decomposition of the oil contained in the cotyledons lose the faculty of germination before the rainy season, in which the ligneous integument of the pericarp opens by putrefaction.'

There are various explanations current as to the way the seeds are set free from their iron-like enclosure; such as that monkeys break the shell by striking it on stones; or that

rodents succeed in gnawing through it; or that the seeds germinate whilst inside the pericarp, and the first seedling to push out the plug and emerge through the aperture occupies the whole of it, so as not only to prevent the other seedlings from getting out, but also to provide for its own future in the decayed remains of its brethren, on which it lives until the pericarp has been softened by natural decay.

The arrival at Kew, six years ago, of fresh fruits of *Bertholletia* from Trinidad offered an opportunity to get at the truth of the matter. Accordingly, two of the most perfect were placed, on October 22, 1894, in a bed of moist cocoa-nut fibre kept at a temperature of from 75° to 85° Fahr. They were quite intact, the plugs firmly fixed in the apertures, and it is unlikely that either air or water could find a way in. Some of the fruits received at the same time were broken open, a by no means easy operation. The outside of the pericarp was as hard as well-seasoned oak, whilst the inner lining was even harder, and smooth and glass-like.

After the two fruits had been in the bed a year, on no signs of growth being visible, the shell of one was carefully sawn in half without disturbing the nuts. It was then as hard and water-tight as when it was planted. Germination had begun, and some of the seeds had shoots several inches long. The plug fell out during the sawing operation, and on examining the plug of the second fruit we found that it had shrunk and no longer served to keep out air and light. The cut shell was put together again and bound with wire. It had however been spoilt for the purpose of the experiment, the result of the interference being to hasten the growth of the seedlings and the decay of the pericarp so that a batch of healthy plants resulted.

A seedling pushed through the aperture in the second fruit eighteen months from the time of planting, and it was followed by others until within a few weeks six had found a way through. They grew as well as seedlings under ordinary conditions until they felt the pressure of the ring-like aperture, when the effect was seen in their smaller leaves

and arrested growth. By the thickening of their stems they had completely closed the way to salvation for which they had raced ; the mystery was, therefore, how they were sustained ; they received no help from us. The pericarp was as hard and apparently as impervious to water as when it was first planted.

The struggle for life continued for the next four years. Now and then a plant would die, but its place was soon taken by another shoot, and this went on until the sixth year. Although it appeared that other seedlings were waiting inside until the death of one of those outside afforded them a chance, yet it seemed more than doubtful that seeds which had germinated about four years before could be still alive without having ever seen the light. An explanation was forthcoming when, on finding the pericarp had softened so that it could be broken away by hand, we decided to open it. This was done on October 22, 1900, exactly six years from the date of sowing.

What we saw is shown by the three photographs here reproduced (Plates IV and V).

I. The fruit with a portion of the pericarp removed, showing the arrangement of the seeds, and the result of the pressure on the lower portion of the stem of the seedlings. Six were alive. The upper part of the stem of one had died, but a new lateral shoot had started again from the base near the seed.

II. The whole of the nuts and seedlings after the pericarp had been removed. The shells of every one of the fifteen nuts which the fruit originally contained were there, and nearly all intact ; five had never germinated, and contained only mould-like remains ; four had germinated, but perished afterwards ; six had formed plants.

III. Three of the seedlings. These show how great the struggle had been : (*a*) has had the life squeezed out of the exposed part of its stem three times, but had still sufficient vigour to start a fourth shoot ; (*b*) bears evidences of hard times in the condition of the lower part of its stem ; (*c*) has

had the best of the fight from the start, and is by far the strongest of the six seedlings.

It will be noticed that (c) has a few woody roots, whilst the other two have scarcely any. The whole of the kernel of the nuts that were successful appears to have formed a tuber-like reserve capable of sustaining growth without any assistance from roots. The six seedlings are now planted singly in pots of soil and show promise of becoming established.

For explanation of Plates IV and V, see text, p. 101.



I

WATSON.—*BERTHOLLETIA EXCELSA*.



II

WATSON.—BERTHOLLETIA EXCELSA.



b

c

a

III

The Embryo-Sac of *Peperomia*.

BY

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With Plate VI.



DURING the summer of 1899 the writer collected at the Royal Botanic Gardens at Kew several species of *Peperomia*, with a view to investigating the development of the embryo-sac and embryo. A preliminary study was made soon after, the more important results of which were afterwards published ¹.

Since the writer's account appeared, the same species has been investigated by Dr. D. S. Johnson², who has confirmed the most important discovery brought out in the former paper—namely, the presence of sixteen nuclei in the unfertilized embryo-sac; but he at the same time called attention to an error in the interpretation of the structures found in the older embryo-sac.

¹ Die Entwicklung des Embryosackes von *Peperomia pellucida*. Ber. der Deutschen Botanischen Gesellschaft, Bd. xvii, Heft 10, 1899. A preliminary note appeared in the Annals of Botany for December, 1899.

² On the Endosperm and Embryo of *Peperomia pellucida*; Bot. Gazette, xxx, No. 1, July, 1900.

It seemed desirable, therefore, to examine the subject again, and a number of new preparations were made, to supply certain stages which were lacking in the series of slides made for the preliminary examination. In preparing these, Flemming's triple stain was employed, which gave much better results than the simpler aniline-water safranin, which had been used before. From a study of these new preparations it was soon evident that Johnson's conclusions were substantially correct, although there were one or two minor points with which the writer's results do not agree.

In the former paper, the writer asserted that no endosperm was formed, and that in the ripe seed the embryo filled the whole embryo-sac. The error arose from a failure to find the stages between the very young embryo, and the stage where the embryo apparently filled the whole sac. The embryo is small, and its cells closely resemble those of the large-celled endosperm. As Johnson has shown, certain of the nuclei take no part in the endosperm-formation, and these being seen crowded against the walls of the embryo-sac by the globular mass of large-celled endosperm, the latter was mistaken for an embryo, which had filled up the embryo-sac, without any endosperm being formed.

THE FLOWER.

The flower in *Peperomia* is exceedingly simple, the gynoecium consisting of a single carpel with a solitary erect ovule, having but a single integument. Two stamens are present, and the flowers, which are set on thick spadix-like spikes, are each subtended by a peltate bract.

The development of the flower is easily followed in longitudinal sections of the young spike, and it was found to agree with the account given by Schmitz ¹.

The ovule develops a single large archesporial cell ², from which a single tapetal cell is cut off, which undergoes repeated

¹ Die Blütenentwicklung der Piperaceen; Hanstein, Bot. Abhandl., ii, 1, 1872.

² Campbell, loc. cit., Figs. 1, 2.

division, and gives rise to several (3-4) layers of cells between the apex of the embryo-sac and the epidermal layer of the nucellus (Fig. 1, *t*).

The inner archesporial cell becomes at once the mother-cell of the embryo-sac.

THE EMBRYO-SAC.

The most remarkable fact brought out in the study of the development of the embryo-sac was the behaviour of the nuclei, which differ remarkably from those of other Angiosperms. The marked polarity of the typical embryo-sac was found to be entirely wanting, and the nuclei were uniformly distributed. Most important of all was the further division, unique so far as is at present known among Angiosperms, of each of the eight nuclei, so that there are normally sixteen nuclei in the unfertilized embryo-sac (Pl. VI, Fig. 1).

The sixteen nuclei are at first entirely similar, and equally distributed in the rather thick cytoplasmic layer which surrounds the large central vacuole. As the embryo-sac develops, a certain number of the nuclei, usually but not always eight, approach, and about them there is an accumulation of cytoplasm (Figs. 2, 3, *E. n*). This group of nuclei is often placed at the chalazal end of the embryo-sac, and much resembles a group of antipodal cells, although, as we shall see, they are not the homologues of the antipodals of the typical Angiosperm embryo-sac.

At the micropylar end there may be found two or three, or occasionally more, nuclei, while the remainder are arranged without definite order about the wall of the embryo-sac. Of the nuclei at the upper end of the sac, one soon becomes somewhat larger than the others, and there is an accumulation of cytoplasm about it, which is bounded by a more or less evident protoplasmic membrane (Figs. 3, 4, *o*); but this is not so evident, nor is the egg-cell thus formed so large, as Johnson describes for his preparations. This, together with some other slight variations, suggested that perhaps his plant

was not the same as the one cultivated under the same name at Kew.

The behaviour of the other nuclei, at the apex of the sac, is not always the same. In some cases observed, where there were two of these, they were arranged with reference to the egg-cell in such a manner as to suggest the typical angiospermous egg-apparatus. Johnson¹ states that he always found a single conspicuous synergid, which after fertilization developed a cell-wall and persisted until the embryo was nearly full-grown. The writer found, in a good many cases, preparations which showed an appearance not unlike that described, but it was impossible to see in what essential respect the so-called synergid was different from the other nuclei which do not form part of the group already referred to.

These also develop a membrane about them, and are often quite as conspicuous as the alleged synergid (Fig. 6, *B*, *x*) which we are inclined to think is nothing more than one of these nuclei with its accompanying cytoplasm, which simply is close to the egg. As there may be two, or even three of these cells in the upper part of the embryo-sac, and they take no part in the conduction of the generative nucleus to the egg, it is very questionable whether they can be properly spoken of as synergids.

At the time the pollen-tube reaches the embryo-sac, the egg-cell, which may be slightly pushed to one side, appears as a flattened body, and close to it there are from one to three nuclei, with a more or less definite aggregation of cytoplasm about them, and perhaps to be considered as the morphological equivalents of the synergidae of the typical embryo-sac. They take no part, however, in the process of fecundation.

The group of (usually) eight nuclei already referred to (Figs. 2, 3), may at the time of fertilization occupy the base of the sac, or these nuclei may be aggregated at a point on one side, or even close to the egg-cell. These, as Johnson has

¹ loc. cit., p. 2.

shown, are the homologues of the two polar nuclei of the ordinary Angiosperms.

The remaining nuclei, which lie close to the wall of the embryo-sac, are more or less flattened, and subsequently develop a cell-wall about the deeply stained cytoplasm, forming a lenticular cell which projects into the cavity of the embryo-sac (Figs. 5, 7, *x*).

THE POLLEN.

The small, globular pollen-spores (Fig. 8) have a roughened membrane, and contain when ripe two relatively large nuclei. The larger of these, which stains much less deeply than the other, and has a more conspicuous nucleolus, is the vegetative nucleus. The other, which stains very strongly, is the generative nucleus. In no case was a division of the latter observed in the ungerminated spore.

Pollination is effected some time before the embryo-sac is mature. Nothing unusual was noted in regard to the growth of the pollen-tube through the pistil. It penetrates as usual through the micropyle, and on reaching the apex of the nucellus pushes apart the large epidermal cells, and then crowds between the tapetal cells, which are not, however, destroyed, and finally comes into direct contact with the egg (Fig. 3).

POLLINATION.

In one case (Fig. 4) two similar, deeply stained nuclei were seen within the end of the pollen-tube which had just reached the embryo-sac. These were, with little question, the two generative nuclei, which were the result of the division of the single generative nucleus of the ripe pollen-spore. The vegetative nucleus of the pollen-spore could not be certainly detected in any instance within the pollen-tube, although in one case a body that looked as if it might be the disorganized remains of a nucleus was seen within the tube, above the generative nuclei.

FERTILIZATION.

Although repeated search was made, it was not possible to determine the fate of the second generative nucleus. One enters the egg directly from the pollen-tube, but what becomes of the other is not clear. The nuclei of the cells immediately surrounding the embryo-sac appear contracted and deeply stained in the preparation, and although in several cases a nucleus was seen which looked as if it might be the second generative nucleus, it was not possible to be certain whether this did not belong to one of the disorganized cells of the inner nucellar tissue.

In the specimen shown in Fig. 3, the generative nucleus had apparently just entered the egg-cell. The nuclear membrane appeared indistinct, and the chromatin formed an irregular mass, somewhat suggestive in form of a spermatozoid; but as no others were found in this stage, it must remain undecided at present, how far this is a normal appearance. Later, as Johnson has shown, the male nucleus assumes a form closely resembling that of the egg-nucleus, except that it is somewhat smaller; but it is always decidedly larger than the generative nucleus of the pollen-spore (Figs. 5, 10).

The fusion of the two nuclei does not usually occur at once, but there seems to be a good deal of difference in the time in different instances. In one case observed, the fusion of the nuclei was completed, and the first division of the embryo ensued, before any division of the endosperm took place; but usually the fusion of the sexual nuclei is not accomplished until after the completed fusion of the endosperm nuclei.

As usual in Angiosperms, the two sexual nuclei become closely appressed (Figs. 5, 10); and finally the cavities of the two are thrown together, and a single nucleus results.

THE ENDOSPERM.

As we have seen, the eight endosperm-nuclei lie close together, often actually in contact, and surrounded by a mass of cytoplasm which is much thicker than the layer lining the

embryo-sac at other places. This group of endosperm-nuclei may be either at the base of the sac, or at the side, even close to the fertilized egg. Shortly after fertilization is completed, the cytoplasm within the embryo-sac increases greatly in amount, and the central vacuole disappears. At this stage (Fig. 6) the cytoplasm is usually densest in the centre of the sac, and surrounding this central mass are numerous smaller vacuoles separated by thick layers of granular cytoplasm. The group of endosperm-nuclei usually occupies the central part of the cytoplasm (Fig. 6).

The fusion of the group of endosperm-nuclei, to form the definitive nucleus, which has been followed in detail by Johnson¹, could only be found in its earlier phases (Fig. 7); but there seems no reason to doubt the accuracy of his observations, as the single endosperm-nucleus resulting from the fusion of the group was found, and its division observed. According to Johnson's account, two of the nuclei first fuse, and this is followed by the gradual absorption of the whole group into one very large nucleus, in which the nucleoli are more or less completely fused.

Fig. 9 shows the large endosperm-nucleus, apparently in the early prophase of division. The form was somewhat irregular, showing evidences of its compound structure. The nucleoli had quite disappeared, and the very numerous chromosomes were plainly evident and showed an almost complete separation into two groups, almost suggesting a direct division of the nucleus. It is certain, however, that a typical mitosis occurs in some cases, and it is not likely that a direct division of the primary endosperm-nucleus ever takes place, although the possibility of such a division is not to be denied.

The first division-wall in the endosperm is usually vertical (Fig. 10), but it may be more or less inclined, or even almost horizontal. The resting nuclei after the first division (Fig. 10) are very large and contain numerous nucleoli, each of which, in stained specimens, shows a clear area about it: they have

¹ loc. cit., pp. 4, 5.

very much the appearance of being made up of several fused nuclei.

The dense cytoplasm of these cells shows the coarse, vacuolate appearance seen in the undivided embryo-sac. The subsequent division-walls are mostly radial, so that the young embryo is surrounded by a single layer of very large endosperm-cells. The contents of these cells become more uniformly granular as they grow older, and the vacuoles mostly disappear. Later, there may be formed a small number of periclinal divisions; but even in the ripe seed many of the endosperm-cells extend from the embryo to the periphery of the embryo-sac. Johnson states that there may be forty or more endosperm-cells in the ripe seed.

THE ACCESSORY NUCLEI.

The nuclei which do not fuse to form the endosperm-nucleus are usually closely appressed to the wall of the embryo-sac, but this is not always the case. In Fig. 6, *B*, is shown one of these nuclei which has remained very like the endosperm-nuclei, and has surrounded itself with a cell-wall, forming a very conspicuous cell projecting into the embryo-sac. In this case there was a similar conspicuous cell occupying the position corresponding to the single synergid described by Johnson; and, as the accessory nuclei regularly develop a cell-wall, become filled with deeply stained cytoplasm, and are very similar to the supposed synergid, it is probable they are homologous with it.

While the group of unfused endosperm-nuclei, with the surrounding cytoplasm, often occupies the antipodal end of the embryo-sac and looks very much like a group of antipodal cells, their subsequent behaviour shows that they are not the equivalents of the antipodals of the typical angiospermous embryo-sac. These must be sought in the separated cells, developed about the accessory nuclei, which here are not united in a group, but are distributed singly about the periphery of the embryo-sac. While they can hardly be spoken

of as 'antipodal' cells, they must be regarded as their equivalents, being those nuclei which are not directly concerned in the production of the egg-apparatus or endosperm.

THE EMBRYO.

The embryo remains very slightly developed, even in the ripe seed. The fusion of the two pro-nuclei is often not complete until the endosperm-nuclei have been fused. The fertilized egg-cell has grown somewhat and become almost globular. The first division is longitudinal, and is followed by a second longitudinal wall in each of the two cells. Transverse walls then form, but the embryo increases but little in size, nor is there much enlargement of the embryo-sac after the endosperm begins to develop. In *Peperomia*, as in all the Piperaceae, the nutritive function of the endosperm is assumed by the perisperm, developed from the nucellar tissue, whose cells become filled with starch.

THE FRUIT.

The development of the fruit was not studied in detail, but as the writer's observations did not agree in all respects with those described by Johnson, a brief note of these differences will be given.

From the first, the ovule completely fills the ovarian cavity, and finally a sort of caryopsis is formed. The innermost layer of cells of the ovary-wall becomes very large, and upon their membranes are formed conspicuous reticulate thickenings¹, so that this layer forms a hard shell, covered with several layers of thin-walled cells. It is this inner layer of the carpel which constitutes the principal part of the hard portion of the fruit.

The cells forming the two layers of the integument become very dark-coloured, but this is mainly due to a change in the character of the wall. They stain so strongly as to appear quite opaque, but in thin sections it may sometimes be seen

¹ Johnson, loc. cit., Fig. 15.

that the cell-cavity is not closed, as Johnson describes (Fig. 13). The inner layer of integument-cells has the internal walls strongly undulated, so as to crowd and distort the outer layer of cells of the nucellus, which Johnson describes as belonging to the integument, supposing apparently that the darkly stained inner layer of cells of the integument was simply the much thickened cell-wall, belonging to what was really the outer nucellar layer¹. The real condition is readily seen in the younger fruits (see Figs. 12, 13).

SUMMARY OF RESULTS.

1. All species of *Peperomia* seem to agree in having regularly sixteen nuclei in the embryo-sac instead of the eight normally present in other Angiosperms. There is no trace of the marked polarity usually observed in Angiosperms.

2. One of the nuclei in the micropylar end enlarges somewhat, and there is an accumulation of cytoplasm about it to form the egg-cell. From one to three other nuclei are found near the egg-nucleus, and these may show a more or less evident aggregation of cytoplasm about them, and may perhaps be regarded as the equivalents of the synergidae of the ordinary egg-apparatus. They take no part in fertilization, and their synergidal character is open to question.

3. Several (usually eight) nuclei fuse to form the endosperm-nuclei, but must be considered as the homologues of the polar nuclei of the typical Angiosperms. The remaining nuclei are scattered, and each develops about it a cell-wall, much as do the antipodal cells of many Angiosperms.

4. The embryo remains very small, and shows no differentiation when the seed is ripe.

5. The divisions of the endosperm-nuclei are always accompanied by the formation of cell-walls.

6. The hard coat of the fruit is mainly formed from the innermost layer of cells of the pericarp.

¹ loc. cit., p. 7, Figs. 14, 15.

THEORETICAL CONSIDERATIONS.

The writer has already expressed his opinion that in *Peperomia* we have to do with the most primitive type of Angiosperm yet described. Johnson is inclined to differ from this conclusion, believing that the peculiarities are to be explained as secondary modifications. His reasoning is not, however, quite convincing. He says¹, 'I am inclined to believe that the peculiarities of the embryo-sac of *Peperomia* have been secondarily acquired, and are analogous to those found in Angiosperms of peculiar habit, e.g. many aquatic, parasitic and saprophytic forms.' Now the habit of *Peperomia*, so far as the writer is aware, conforms to none of the above categories, and is that of any normal green plant. Moreover the peculiarities of the embryo-sac are *not* reductions—unless we consider the embryo, which is not the question here; but consist rather in an increase in the number of parts, for which there is no parallel, so far as the writer is aware, as a result of the aforesaid peculiar habits.

Piper and *Heckeria* are undoubtedly related to *Peperomia*; but *Saururus* certainly cannot be considered a very near relative, as Engler thinks it sufficiently distinct from the Piperaceae to warrant the establishment of a separate family. Moreover the flowers, with their syncarpous gynoeceum, are certainly much more specialized than the exceedingly simple unicarpellate flower of *Peperomia*. Just why the flower in *Peperomia* should be considered as a reduced form is not clear. From a study of many low types among the Monocotyledons, which cannot be readily derived from higher types, e.g. *Naias*, many Araceae, it seems more reasonable to consider the single carpel, with a single axial ovule, as the primitive type for the Angiosperms, and with this *Peperomia* agrees perfectly.

While Johnson states that *Piper* and *Heckeria* have 'essentially typical' Angiospermous embryo-sacs, he gives no details or figures, so that it is impossible to judge whether

¹ loc. cit., p. 9.

there may not be some points in their structure which might be compared with some of the stages of development in *Peperomia*. That *Saururus*¹ should show a typical angiospermous embryo-sac is to be expected, as it does not exhibit any especially primitive features in the flowers, and is distinctly more specialized in this respect than are the Piperaceae.

As regards the significance of the fusion of the endosperm-nuclei, we agree with Johnson and Strasburger² that it is not a true fertilization, but has a nutritive significance only; and the whole endosperm, as well as the antipodal cells or their equivalents, represent gametophytic structures, and the endosperm is not to be considered as an embryo.

The behaviour of the endosperm-nuclei in *Peperomia* is a strong argument in favour of its primitive character, and is what might be expected from its other peculiarities. In harmony with the generalized character of the other structures of the embryo-sac, there occurs, instead of the two definite polar-nuclei of the other Angiosperms, a fusion of a somewhat variable number, which cannot be seen to bear any definite relation to any of the primary nuclei of the sac. With the reduction by half in the number of the embryo-sac nuclei, and the strongly marked polarity observed in most Angiosperms, the number of these nuclei becomes reduced to two, the polar nuclei, and the antipodal cells assume their characteristic position.

In regard to the homologies of the structures in the embryo-sac, the writer believes that the contents of the embryo-sac with the sixteen nuclei represent a prothallial tissue, and the nuclei are at first entirely similar. One of these becomes differentiated to form an archegonium, which is reduced to a single cell—the egg-cell. Whether the adjacent nuclei are to be considered as synergidal nuclei

¹ Johnson, D. S., On the Development of *Saururus cernuus*, L., Bull. of the Torrey Bot. Club, July 27, 1900.

² Einige Bemerkungen zur Frage nach der 'doppelten Befruchtung' bei den Angiospermen. Bot. Zeit., 1900, p. 293.

or not is doubtful, and the question whether the synergidae are, so to speak, sterile archegonia, or whether they are simply vegetative prothallial cells which become specialized for assistance in fertilization, cannot now be answered. The nearest approach to the structures found in *Peperomia* occurs in *Gnetum*¹, where no archegonium is formed, but the generative nucleus from the pollen-tube fuses with one of the free nuclei in the upper part of the embryo-sac.

That the fusion of the polar nuclei of the typical Angiosperms is in no sense a sexual process is borne out by the condition of affairs in *Peperomia*. The fusion of such a mass of nuclei is not conceivable as a sexual process, and is with little question, as already stated, a nutritive process, or perhaps a stimulus to active division in the endosperm-formation, or secondary growth of the prothallial tissue, which is to nourish the young embryo. This has no equivalent among either Archegoniates or Gymnosperms, and until further evidence is offered, may be assumed to have arisen among the lower Angiosperms, and to have become restricted to the special polar nuclei as the number of nuclei was reduced from sixteen to eight. The fusion of the second generative nucleus with the complex must also be assumed to be a special development. The second generative nucleus discharged into the embryo-sac, not having an egg with which to conjugate, might very naturally fuse with the only available nucleus, i. e. the endosperm-nucleus. That the tissue arising from this compound nucleus should show evidences of its hybrid character, where cross-pollination has taken place, is what would be expected, and by no means implies that the hybrid endosperm is in any proper sense of the word an embryo. The small cells developed individually about the nuclei which do not participate in the endosperm-formation in *Peperomia*, while they are not grouped together, must nevertheless be regarded as equivalent to the antipodal group of the typical Angiosperms, where the position of these cells

¹ Lotsy, Contributions to the Life-history of the Genus *Gnetum*. Ann. du Jar. Botanique, Buitenzorg, xvi, 1900, p. 46.

at the antipodal end is correlated with the marked polarity of the embryo-sac structures. In short, both antipodal cells and endosperm are equally of prothallial nature.

From a study of the condition of things in *Peperomia*, there is not the slightest evidence either that the antipodal group of the typical embryo-sac represents a second egg-apparatus, or that the polar nuclei are sexual in their nature.

THE SYSTEMATIC POSITION OF PEPEROMIA.

Through the kindness of Dr. D. H. Scott, several species of *Peperomia* were germinated at Kew, and an examination of these showed them to be true Dicotyledons—of which group *Peperomia* may be considered to be the most primitive type.

The reasons for this conclusion lie of course mainly in the character of the embryo-sac and the extremely simple flowers; but there are other features which point to this genus as a primitive generalized type. *Peperomia* shows several significant resemblances to the lower Monocotyledons, especially the Araceae, which themselves give evidence of being a very low type. The structure of the flowers, and their arrangement upon a thick spike, in many species quite like a true spadix, is noteworthy, while the habit of the plant, the form of the leaves, and the arrangement in the stem of the vascular bundles recalls very strongly the structure of the Araceae.

Moreover, the endosperm-formation is very similar in the two, being by direct division.

The relation of *Peperomia* to the other Piperaceae is unmistakable, and it is to be hoped that further investigations of members of this family may reveal some intermediate forms. The Saururaceae seem to represent a distinctly more specialized type than the Piperaceae, from which they have probably been derived. There seems no valid ground for supposing that the reverse is the case, as has been assumed by Engler¹,

¹ Engler, Piperaceae, Die nat. Pflanzenfamilien, iii. Th., 1. Abt., 1899.

who apparently regards the simple flower of the Piperaceae as reduced from the type found in the Saururaceae.

The question as to the relative antiquity of Monocotyledons and Dicotyledons is one which has not yet been answered, as the geological record is by no means complete. The close resemblances, however, between the Piperaceae and the lower series of Monocotyledons suggests that the divergence of the two divisions of the Angiosperms may have occurred very early; but much more evidence is necessary before it will be possible to decide this important question.

STANFORD UNIVERSITY, CALIFORNIA,
Jan., 1901.

EXPLANATION OF FIGURES IN PLATE VI.

Illustrating Prof. Campbell's paper on *Peperomia*.

All figures refer to *Peperomia pellucida*, Kunth, and were drawn with the camera lucida, Leitz oil im. $\frac{1}{18}$, oc. I. \times about 600.

Fig. 1. Longitudinal section of an embryo-sac with sixteen nuclei, of which three are at the micropylar end. *t*, the tapetum.

Fig. 2. An older stage, in which the egg-cell, *o*, is differentiated, and the endosperm-nuclei, *E. n.*, are grouped together. A second large nucleus lay close to the egg, but there was no definite synergid.

Fig. 3. Embryo-sac just after the entrance of one of the generative nuclei, *Sp*, into the egg-cell, *o*; *p. t.*, the empty pollen-tube. The endosperm-nuclei, *E. n.*, are collected at the antipodal end of the embryo-sac. Two synergidae (?).

Fig. 4. *A*. Embryo-sac just before the entrance of the pollen-tube, *p. t.* The two generative nuclei can be seen near its extremity. Three synergidae (?); two shown in *B*.

Fig. 5. Fertilized embryo-sac, showing the two pro-nuclei, *Sp, o*, in process of fusion. The endosperm-nuclei were also beginning to fuse. *x*, one of the accessory nuclei, enclosed within its cell.

Fig. 6. *A* and *B*. Two sections of an older embryo-sac, in which the cytoplasm fills the cavity. The two pro-nuclei were still separate. Nine endosperm-nuclei occupied the centre of the sac. In *B* is shown an unusually large accessory nucleus, *x*, enclosed in a nearly globular cell.

Fig. 7. Beginning of the fusion of the endosperm-nuclei, *E. n.* *Em*, one-celled embryo. *x*, accessory nucleus.

Fig. 8. Section of a ripe pollen-spore, showing the two nuclei, *v* and *g*.

Fig. 9. Embryo-sac, shortly before the division of the fusion-nucleus, *E. n.* *Em*, embryo.

Fig. 10. Embryo-sac, after the first division of the endosperm. *Em*, embryo; the pro-nuclei in process of fusion.

Fig. 11. Older embryo-sac, containing a four-celled embryo, *Em*, and several endosperm-cells. *x*, accessory nucleus.

Figs. 12, 13. Longitudinal sections through the integument, *in.*, of the young seeds, showing the peculiar form assumed by the cells of the inner layer. The walls of these cells stain very strongly, but do not become noticeably thickened.

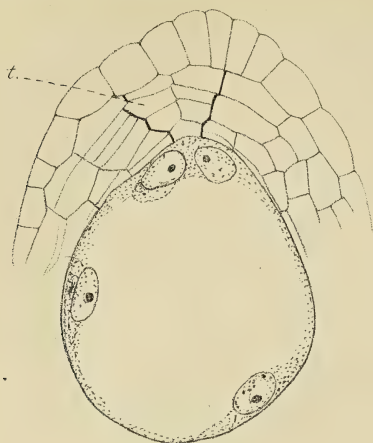


Fig. 1.

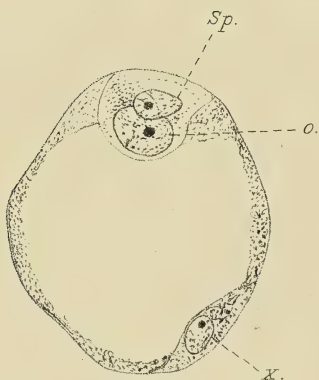


Fig. 5.

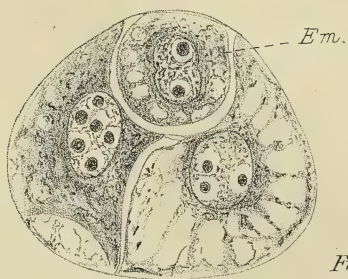


Fig. 10.

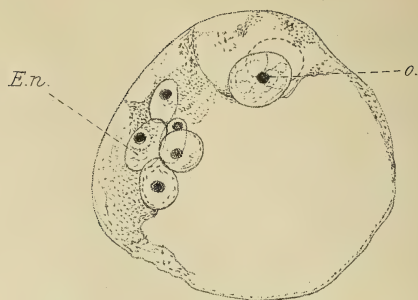
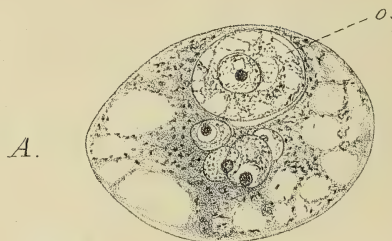


Fig. 2.



A.

Fig. 6.

B.

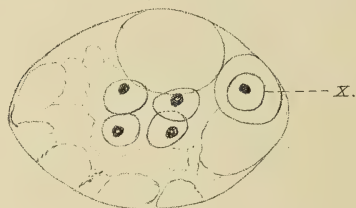


Fig. 11.



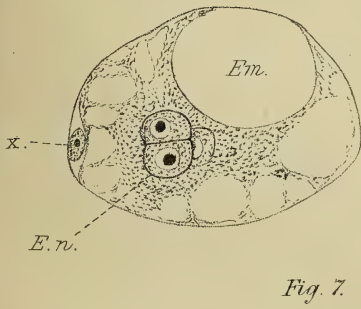
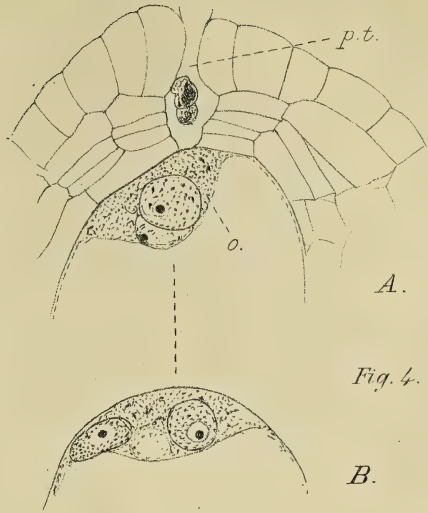
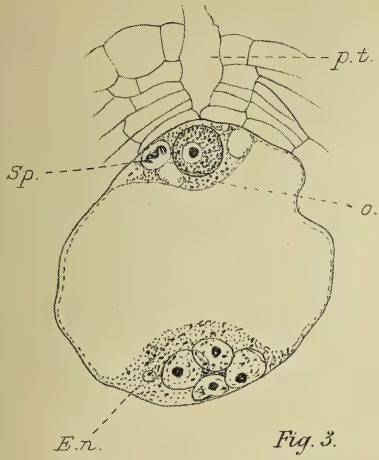


Fig. 9.

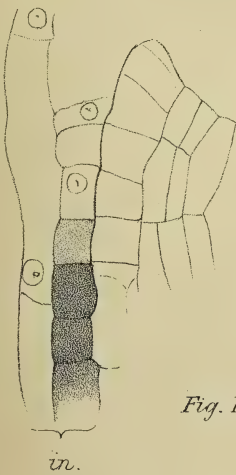
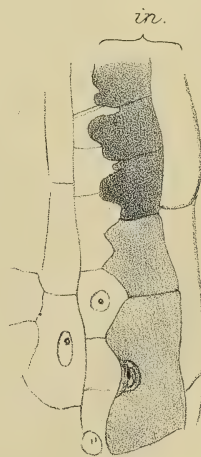


Fig. 13.



On the Biology of *Bulgaria polymorpha*, Wett.

BY

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in the University of Cambridge.*



With Plate VII.



*B*ULGARIA POLYMORPHA in the neighbourhood of Cambridge is usually to be found on the bark of oak-trees which have been felled and allowed to lie in the woods for a year or two, but it has been recorded on living beech by Massee¹, and again by Hennings on living oak². Ludwig even considers it as a dangerous parasite on the oak³. As a rule the Fungus is a saprophyte, but as is so often the case, it is at times capable of becoming parasitic. De Bary calls such Fungi facultative parasites.

The Fungus has been described under the name of *Peziza polymorpha* by Oeder, *Peziza inquinans* by Persoon, *Bulgaria inquinans* by Phillips and Saccardo, and *Bulgaria polymorpha* by Wettstein⁴.

¹ Massee, Textbook of Plant Diseases, 1899, p. 162.

² Hennings, Zeitschrift für Pflanzenkrankheiten, iv, 266, 1894.

³ Ludwig, Centr. für Bakt., 2. 521 and 3. 633.

⁴ Oeder, Flor. Dan., t. 464 (1768); Persoon, Syn. Fung., p. 631 (1801); Phillips, Brit. Disc., p. 314, 1887; Saccardo, Syll., viii, n. 2625; Wettstein, Zool.-Bot. Verh., 1886, p. 595. For figures see Rehm, Krypt.-Flora, Discomycetes,

I quote the description given by Masee¹: 'Ascomphores gregarious or caespitose, erumpent, at first more or less clavate, closed, rusty-brown, and scurfy, the disc gradually expanding and becoming plane or slightly convex, black and shining, externally umber-brown, wrinkled and scurfy, turbinate and narrowed into a short, stout, stem-like base, which is mostly buried in the substance of the host, 1-4 cm. across, and the same in height, gelatinous, flesh brown; asci cylindric-clavate, narrowed below into a long slender base, usually only containing four perfectly developed spores; spores 1-seriate, continuous, elliptical slightly curved, 1-2 guttulate, brown at maturity, 10-14 × 5-6 μ ; paraphyses slender, slightly thickened, brownish, and more or less curved at the tips.'

In its gelatinous texture *Bulgaria* resembles the Tremellineae to such an extent, that a very similar species, *B. sarcoides*, was for some time considered to be a *Tremella*, and when the ascomycetous nature of the Fungus was proved it was considered by some to be a form connecting together the Discomycetes and Tremellineae.

The morphology of *B. polymorpha* was worked out in some detail by Tulasne², and served him as a good example of a pleomorphic species at a time when little was known of the various spore-forms of the Ascomycetes. By observing specimens at different periods of the year Tulasne was able to show that at one time they produced pycno-conidia and spermatia, and then ascospores, which on germination gave rise to a conidial stage; that is, one and the same Fungus was capable of giving rise to four essentially different sets of spores. Brefeld³ has further studied the germination of the ascospores and the conidia formed from them.

p. 472; Tulasne, Ann. d. sci. nat., 3^e sér., tom. xx, p. 164; Brefeld, Unters. aus d. Gesamtgeb. d. Mykol., 1891, Heft x, Ascomyceten, ii; Hussey, Illustr. Brit. Myc., Pl. XXXII.

¹ Masee, Brit. Fung. Flora, vol. iv, p. 140, 1893.

² Tulasne, Ann. d. sci. nat., 3^e sér., tom. xx, p. 164, 1853; Tulasne, Carpologia, tom. iii, p. 192, 1863; Tulasne, Bot. Zeit., p. 54, 1853.

³ Brefeld, Heft x, Ascomyceten, ii, p. 301.

My chief object in examining the biology of this Fungus was to observe the effects of its action on wood, but as the cultures afforded a good opportunity, the course of development was also traced in some detail.

The ripe ascophores used for this investigation were gathered on December 5, 1898, and a further supply was obtained in December, 1899. They are also said to occur during the summer months, but so far I have not found them except in the winter.

An examination of longitudinal sections of the ascophore showed that it was traversed by dark brown veins, which anastomosed immediately below the hymenium (Pl. VII, Fig. 1). Under the microscope the veins were found to be channels bordered by hyphae with thin dark brown walls, while the mass of the ascophore consisted of hyphae with strongly swollen walls. To such an extent was this swelling carried that the walls formed a solid gelatinous mass.

The hymenium consisted of delicate whip-like paraphyses and asci characterized by containing two apparently different forms of ascospores—a comparatively rare occurrence in the Ascomycetes, for all eight spores are usually similar to one another. In this case four had thick dark-brown walls and a deep longitudinal infolding on one side, while the remaining four were colourless and thin-walled. The position of the smaller spores was variable, at times they were crushed into the apex of the ascus or scattered irregularly among the large brown spores. Occasionally, but very rarely, all the spores had thick brown walls. The apex of the ascus was coloured blue by an iodine solution.

On placing the ascospores in beer-wort gelatine they germinated within twenty-four hours (temp. 14°C.), and rapidly gave rise to a dense mycelium. Both the coloured and colourless spores behaved in this way, and beyond the fact that the colourless spores germinated first I could find no difference between them. In about four days the walls of the mycelium began to swell, the swelling starting from the spore and gradually working its way along to the younger

parts. At the same time a bright orange red pigment, the 'bulgarine' of Zopf, was formed within the hyphae. In the case of strongly-growing spores the swelling continued until the diameter of the hypha was increased three or four-fold. Except that a number of fusions occurred in hyphae extending beyond the hanging-drops, possibly as an aid for the transference of plastic substances, nothing further was to be noted in these cultures. This result does not agree with those of Brefeld and Tulasne, who have stated that the spores on germination put out either a rudimentary or a well-developed mycelium from which numbers of rod-shaped conidia were abstricted¹. Further experiments with various media showed, however, that either result could be produced at will. Thus on sowing the spores in distilled water the colourless ones germinated in about twenty-four hours and gave rise to a mycelium about the length of the original spore, from which numbers of conidia were abstricted, while the coloured spores behaved in a similar fashion after two or three days. In a watery extract of oak-wood, or in a gelatine made up with a watery extract of cow-dung, both colourless and coloured spores gave rise to a long, branching mycelium bearing clusters of conidia. It would appear therefore that the formation of the conidia is determined by the supply of available nutriment; where there is little, conidia are produced at once, while where there is plenty, mycelium only is formed.

The conidia themselves swelled considerably on germination and gave rise directly to a mycelium, which on transference to a moist, sterile block of oak-wood developed rapidly and infected it².

The germinating ascospores also are capable of infecting oak-wood directly, for on removing them from beer-wort gelatine to sterilized blocks they soon gave rise to a thick mycelium. As I never succeeded in finding conidia on the

¹ Vide Brefeld, Heft x, Ascomyceten, ii, Plate XI, for figures.

² For details of method, vide Marshall Ward, Phil. Trans., vol. clxxxix, p. 123, 1897.

mycelium on the blocks, I assume that the mycelium from the ascospore is capable of direct infection.

To study the action of the Fungus on wood, sterile blocks of oak and pine-wood were infected from plate-cultures of the ascospores, beer-wort gelatine being used for germinating them in. A week after infecting the blocks it was evident that the Fungus was growing satisfactorily, for the greater part of their surfaces was covered with a thin, tawny yellow felt of mycelium, which had further commenced to spread over the damp cotton-wool plugs on which they rested. The cultures on pine-wood went no further than this, but on the oak-wood the mycelium increased in quantity, turned to a darker brownish-yellow colour, and in three weeks' time small white humps of tissue appeared on them which gradually increased in size and at the same time exuded drops of water (Fig. 2). This secretion of water usually appears to occur when a Fungus is growing rapidly or is making some special effort, as for instance when spores are being produced. As a familiar case I may mention *Polyporus hispidus*, Fr., which while forming its basidiospores exudes quarts of water. The phenomenon is more readily observed in tube-cultures, where evaporation is not so rapid as in the open. It is probably due to the necessity for large supplies of food-stuff, which have to be taken up in solution and the excess of water disposed of.

The development of these white humps proceeded slowly, and it was not until three months had passed that they appeared as the more or less scurfy, gelatinous knobs characteristic of the young ascophores. Externally their appearance was somewhat variable, some were light grey-brown in colour, others deep chestnut or even black. Those growing from the cotton-wool plugs were usually darkest in colour and had a sodden gelatinous appearance. Then the polished black hymenium opened out and in a few days shed its ascospores as a sooty-brown deposit on the sides of the tubes.

Cultures were fixed either by boiling, or with 20% alcohol,

and then taken through the usual series of alcohols before examination for the effects of the Fungus on the wood.

The only noticeable effect on scrubbing off the external mycelium was that the wood was coloured an ochrey-yellow tint. Longitudinal and transverse sections of a block infected a month before fixing showed that all the tissues were thoroughly permeated by hyphae. The large vessels especially were crowded with mycelium, all of which showed the same swelling of the walls. In some cases the entire lumen of a tracheid was blocked by the much-swollen hypha-wall.

At the same time a slight swelling of the thickening layers of the woody elements was visible. On treating transverse sections with Schulze's solution, a few of the most swollen thickening layers gave a deep purple colour—suggesting that the action of the Fungus was one of delignification. In some cases the thickening layers had become detached and invaginated into the tracheids, owing to excessive swelling, in a similar manner to that described by Marshall Ward¹ in the case of *Stereum hirsutum* (Fr.).

Where the swelling of the thickening layers was slight, Schulze's solution no longer gave either a golden yellow or purple colour, but the layers had a peculiar sheen, very like that of phloem-tissues, which soon became very characteristic of the early stages of delignification. This was followed by the gradual appearance of a faint violet and then purple colour. The kinking off into the lumen of the tracheid of the secondary from the primary thickening layer, or of the two together from the middle lamella, was never found to occur before their complete delignification. Meanwhile, as far as Schulze's solution showed, there was little change going on in the middle lamella; for it still stained a deep golden yellow colour, but in preparations from cultures two months old the elements became dissociated from one another, proving that it gradually went into solution.

Longitudinal sections also showed characteristic symptoms. The bordered pits of oak-wood seen in surface view, at some

¹ Marshall Ward, *ibid.*

focal lengths appear to be surmounted by a cross with arms at right angles to one another. As the walls became swollen, one arm disappeared, while the other became an elliptical slit which gradually broadened until it was roughly circular in outline. Hartig¹ figures a somewhat similar series of events in the case of oak-wood attacked by *Thelephora perdix* (Hartig), *Stereum frustulosum* (Fr.).

Schulze's solution, however, swells the walls of the elements to a considerable extent and so obscures many details. To obviate this I have used a saturated aniline-water solution of gentian violet and a saturated 50 per cent. alcohol solution of Congo-red. The sections were stained from sixteen to twenty-four hours in gentian violet and then transferred directly to the Congo-red solution, where they were usually left for an hour and then dehydrated and mounted in Canada balsam.

Tangential longitudinal sections of cultures a fortnight old were then for the most part stained an intense blue, except at pits where hyphae had passed through the walls. These pits were then marked by a sharply-defined, bright pink zone surrounding them, indicating that that portion of the wall had been delignified, and a cellulose basis staining with Congo-red remained.

In the case of cultures a month old this action was very marked, and one could easily find, especially in the vessels, walls in which every pit was marked in this way. Moreover no hyphae passed through the majority of these pits, so that one has to assume the secretion of a delignifying enzyme in quantity by the Fungus into the wood-elements. The rings round the pits gradually increased in size and in time met one another, so that the surface of the wall was marked by a clear blue network with angular patches at the corners of the mesh-work, on a pink ground (Fig. 3). Ultimately these angular patches disappeared also and the whole surface was stained pink owing to the entire delignification of the wall. The corresponding appearances in transverse section were

¹ Hartig, *Zersetzungserscheinungen des Holzes*, p. 103, and Taf. XIII, 1878.

readily traced in preparations from cultures six or eight weeks old. In these all stages of delignification were visible as a rule.

In the portions least attacked the thickening layers of the various elements were still stained an intense blue, except in the immediate region of pits, where the pink staining again indicated delignification, while in the portions most attacked they were pink throughout. The effect of the action of the Fungus on the middle lamella was also beautifully shown. At first the lamella stood out sharply as an intensely blue line, which gradually became thinner and thinner until it entirely disappeared, except at the corners where several cells met. Where the sections had passed through a pit the lamella was wanting on either side for some distance, and if the action had only just started, a lens-shaped patch of delignified tissue was clearly marked off round it (Fig. 4). In still more diseased portions the gradual disappearance of the angular portions of the middle lamella was traceable. They first became hollow owing to the solution of the so-called 'inter-cellular protoplasm' of Russow¹ and then dissolved entirely (Fig. 5). In this stage the various elements were separated from one another and the wood readily crumbled away on sectioning. As far as my cultures go, this entire breaking up of the wood was of very local occurrence. It was never so complete as in the case of *Stereum frustulosum*, for example.

All the woody elements were attacked in precisely the same way, and at about the same period, with the exception of the tracheids of the medullary rays. These resisted the attack for some time and stood out as dark blue bands, in transverse sections, when almost the whole of the lignin had disappeared from the other elements. Ultimately they too were attacked, however, the action starting most frequently at the point where they abutted on a vessel.

The early stages of this localized delignification of the walls resemble the early stages in the destruction of the thick cellulose walls of the endosperm of germinating seeds of

¹ Gardiner, Proc. Camb. Phil. Soc., vol. v, pt. ii.

Tamus communis. Gardiner¹ has shown in these that the cytohydrolytic enzymes travel along the protoplasmic threads traversing the walls and dissolve them in their immediate neighbourhood. The action is most intense near the middle lamella, so that each thread seems to lie in a cone-shaped portion of the partially dissolved wall, the base of the cone resting on the middle lamella. Sections in the plane of the threads would therefore show a more or less lens-shaped patch of partially dissolved cellulose.

Although, as far as I know, no one has yet proved that threads exist in woody walls similar to those we are dealing with, yet it seems probable that they existed while the thickening layers were being deposited, so that there is a possibility that the pits and the walls are traversed by the minute passages they once occupied. If this is in reality the case, we may apply Gardiner's explanation of the destruction of the cellulose endosperm-walls to the destruction of the lignified walls, by assuming that a delignifying enzyme travels along the slender passages formerly occupied by the threads and decomposes the lignin in their neighbourhood. Whether the increased intensity of decomposition near the middle lamella is due to slight differences in the structure of the succeeding strata of the cell-walls or not has still to be proved. At all events, the first appearance of these delignified lens-shaped patches and the gradual and regular spread of the delignification from the pits accord so well with the assumption made as almost to amount to a proof of the existence of protoplasmic threads, or of the passages once occupied by them in woody walls.

So far I have assumed the existence of a delignifying enzyme to explain these results. Such an enzyme has recently been isolated by Czapek² from *Merulius lacrymans* and has been named Hadromase. Before seeing Czapek's paper I had considered that there was evidence for lignin being a glucoside capable of being split into pectic acid and

¹ Gardiner, Proc. Roy. Soc., 1897-98, vol. lxii, p. 100.

² Czapek, Ber. d. deutsch. bot. Ges., 1899, xvii, p. 166.

glucose, a view which is partially supported by the frequency of the occurrence of glucoside-splitting enzymes in wood-destroying Fungi¹. To test this, a number of cultures on oak-wood saturated with five per cent. solutions of glucose, laevulose, maltose, cane-sugar, and xylose were made, on the assumption that where the glucose was already available the Fungus would no longer need to decompose the wood to obtain it, and also to see, if this was indeed the case, whether glucose could be replaced by any other sugar.

These cultures were examined at fortnightly intervals, and at first looked promising, for in every case except that of the glucose-saturated wood the mode of attack was normal. However, after the action had continued for six weeks the glucose-saturated wood showed the characteristic symptoms of attack, although, as subsequent analysis proved, glucose was still present.

At the same time it is worth noticing that the cotton-wool plugs at the base of the tubes usually produced a fine crop of ascophores, so that it might seem that the delignifying enzyme was not absolutely necessary for the welfare of the Fungus, since the plugs and watery extract of wood could contain no lignin. However, on attempting to grow the Fungus on cotton-wool soaked in a watery extract of oak-wood its development was so slight that it became certain that the necessary nutriment had been conveyed in the former case through the mycelium connecting the blocks to the cotton wool. At the present time our knowledge of the constitution of lignin and of the structure of the lignified cell-wall is exceedingly slight². According to Czapek³ the lignified walls are composed of a hadromal-cellulose ester, which under the influence of the enzyme hadromase is split up, leaving a cellulose basis. Thus after treating wood with an aqueous extract of *Merulius lacrymans*, it shows the cellulose reaction when treated with Schulze's solution, while the

¹ Bourquelot, Bull. de la Soc. Myc. de France, tom. viii, p. 13, 1892.

² Vide Green, Sci. Prog., vol. vi, p. 344, for a summary and literature, 1897.

³ Czapek, *ibid.*

extract on concentration gives the usual lignin reactions with phloroglucin and hydrochloric acid.

The results already seen in the decomposition of the thickening layers agree with this inasmuch as a cellulose basis remains after the disappearance of the lignin, but the middle lamella is also delignified and moreover dissolved. It is impossible therefore to extend Czapek's results to include the middle lamella, which, as we know from the researches of Mangin and Kabsch¹, is composed primarily of pectates, and yet is as lignified as, or more so, than the cell-walls. They show us merely that some Fungi have the power of dissolving out lignin as such. We have still to determine how Fungi utilize this presumably valuable food-stuff. That they do so seems certain, for on testing diseased wood with phloroglucin and hydrochloric acid I have never been able to find a trace of the red staining which should accompany unaltered lignin in the mycelium.

The results of the action of *Bulgaria polymorpha* upon oak-wood are, then, to dissolve and probably decompose the lignin, and to dissolve the pectates of the middle lamella. I have never seen any evidence, either in pure cultures or in naturally diseased wood, pointing to further action, as is the case when some Fungi first delignify the wood and then decompose the cellulose which remains. Moreover, the action is too slight in all the cases I have examined to warrant the supposition that the Fungus is capable of causing a really serious tree-disease such as Ludwig assumed.

The development of the ascophore has been traced from the pure cultures grown on oak-wood or on the cotton-wool plugs on which the blocks rested. The results agree with those of Tulasne², except that I have been unable to find any spermatia or spermogonia. When, however, one attempts to completely cultivate Fungi in pure cultures one realizes how difficult it is to successfully imitate the diverse conditions

¹ See previous page, note 2.

² Tulasne, *ibid.* The account given here merely supplements that in the *Ann. d. sci. nat.*

to which the naturally growing Fungi are exposed, and consequently to obtain all the possible spore-forms. I am therefore unwilling to doubt the accuracy of Tulasne's observations, even though my cultures have reached the ascus-bearing stage.

In each case the ascophores were fixed in Keiser's solution and preserved in alcohol. The earliest stage examined is that shown as a white patch in the second figure. It consisted of a loose plexus of hyphae with such strongly swollen walls that no spaces were left between them, traversed here and there by hyphae with thin, dark walls. At the base of this a number of spherical portions were sharply delimited by walls built up of carbonized hyphae. At first they appeared as hollow shells, which in the course of a month became lined with a gelatinous mycelium, from which conidia (the stylospores of Tulasne) were abstricted. After this the conceptacles became full of a gelatinous mycelium, and by their subsequent growth formed the greater part of the ascophore (Fig. 6). Only the extreme upper portion, and fragments of the outer margin, which in part contributed to the formation of the scurfy scales, was derived from the original plexus. These scales were, for the most part, composed of hyphae with numerous septa and carbonized walls. As this rapid growth proceeded, the hyphae of the gelatinous portions became locally swollen into balloon-like outgrowths, which occasionally became carbonized, and then had somewhat the appearance of chlamydospores. This swelling usually began at knee-like bends in the hyphae (Fig. 7). I have only observed this in pure cultures, and have found no trace of it in naturally-growing specimens. The next observable stage was that a dense mass of paraphyses was developing between the original plexus and the still-distinguishable, carbonized walls of the conceptacles. At first they were straight, and then, as growth continued, sigmoidally curved owing to the resistance met with to their upward growth. Their obvious function was to bring about the opening and expansion of the disc.

Owing to the contraction of the gelatinous hyphae when hardened in alcohol and their subsequent expansion when removed to water it was impossible to obtain a series of microtome-preparations, so that the search for antheridial and oogonial branches had to be abandoned. The early stages in the development of the ascus itself were easily observed, and as they were found to accord with those described by Harper¹, Dittrich², and Gjurasin³, need no further description.

In the young ascus the apex was appreciably thickened, and when treated with iodine solution a cylindrical plug, hollowed out towards the interior, was differentiated by staining a deep blue. As it increased in size the plug became stretched, and could only be distinguished with difficulty.

When the eight spores were first formed it was impossible to say which four would become thick-walled. Each had a single central nucleus which divided into two, and the daughter-nuclei travelled to the poles, while a large vacuole formed between them. Staining at this stage however, with Weigert's haematoxylon or with safranin-gentian violet, showed that some change was occurring, for four of the spores stained deeply, while four remained practically colourless, except at the poles. This was to be explained by the formation of a carbonized wall, almost impermeable to stains, round the developing spore. The brown-walled spores then increased slightly in size, and the deep longitudinal depression, giving the spore its characteristic appearance, developed. The colourless spores did not increase in size after the division of the original nucleus.

Lately Wisselingh has shown that the walls of fungal hyphae in the majority of cases consist of chitin, so that it became of interest to determine whether this was the case for the gelatinous walls met with in *Bulgaria*. On

¹ Harper, Ann. of Bot., xiii, p. 467, 1899.

² Dittrich, Cohn's Beitr., viii, p. 17, 1899.

³ Gjurasin, Ber. d. deutsch. bot. Ges., xi, p. 113, 1893.

adding an iodine solution, especially to sections of young ascophores, the gelatinous portion gave a pale blue or violet colour, while Schulze's solution coloured it a faint violet lavender. The bluish colour was often emphasized by the bright golden-staining contents of some of the hyphae. On heating the sections in sealed tubes with concentrated caustic potash, and then treating with sulphuric acid and iodine, every portion gave the characteristic rose-red colour of the chitin test¹, so that the carbonized and gelatinous walls and the plug at the apex of the ascus have all a chitin basis.

The early stages in the development of the ascophore remind one to a certain extent of the life-history of some of the Pyrenomycetes, such for example as the Valsaceae or the Diatrypaceae among the Sphaeriaceae². In these cases pycnoconidia (stylospores) are formed in conceptacles with walls of carbonized hyphae embedded in a stroma, and later these same conceptacles give rise to asci, and are then known as perithecia.

In *Bulgaria polymorpha* we also find a stroma in which pycnoconidia are developed, so that if the homology be granted, its ascophore consists of a number of perithecia welded together by subsequent growth (Figs. 1 and 8), while the stroma is reduced to the loose hyphae traversing the 'veins' and the apical portion of the ascophore which at first roofs over the hymenium. The hymenium itself in this case might be described as an overflow from the perithecia, the asci and paraphyses being carried out by the great development of gelatinous tissue in the perithecia, to form a regular layer above them instead of a number of isolated groups. Supposing, by way of another example, a series of similar changes to occur in a *Xylaria*, as a result we should have a *Geoglossum*.

Additional interest to this comparison of *Bulgaria* and a *Sphaeria* is afforded by the fact that in both cases the

¹ Wisselingh, Prings. Jahrb., p. 619, 1898.

² Tulasne, Carpologia, tom. ii, p. 97 and p. 212, 1863.

development is to a great extent carried on under the bark of the trees they grow on, while in the higher forms of the Discomycetes the apothecia are always formed superficially on the substratum. It is conceivable therefore that some such development has occurred as an aid to spore-distribution, the flattened exposed disc offering far greater facilities for dispersal than the almost closed and sunken perithecia.

On the other hand, in our ignorance of the phylogeny of the Ascomycetes, we may speculate as to whether the Sphaeriaceae have not developed in the opposite direction also, with the result that the perithecia with partially suppressed walls have become completely enclosed in the stroma. Such a structure would be very like those we meet with in the *Tuberaceae*.

EXPLANATION OF FIGURES IN PLATE VII.

Illustrating Mr. Biffen's paper on *Bulgaria*.

Fig. 1. Longitudinal section through a ripe ascophore to show the 'veins.'

Fig. 2. A culture of the Fungus on oak-wood between three or four weeks old. The light patches are the earliest visible stages of the developing ascophores. $\times 1$.

Fig. 3. Pitted walls of oak-wood seen in surface view, showing various stages of delignification. Near the centre of the figure the pits have only recently been attacked, as shown by the narrow delignified zone surrounding them. These zones extend until they meet, as in the top left-hand corner, and ultimately the whole wall becomes delignified. $\times 375$.

Fig. 4. Transverse section of oak-wood to show the earliest stages in its decomposition. The delignified lens-shaped patches are to be associated with the position of 'pit-threads.' $\times 600$.

Fig. 5. A similar preparation to show the dissolution of the middle lamella. The central cell shows the middle lamella in its normal condition on the right-hand side, while almost complete dissolution is shown in the right-hand bottom corner. The solution of the 'intercellular protoplasm' has just become visible. $\times 600$.

Fig. 6. A longitudinal section of an ascophore about 2 mm. high, in which the conceptacles are developing rapidly to form the main mass of the ascophore. The dark portions indicate the position of carbonized hyphae.

Fig. 7. A portion of a similar section in detail. The hyphae have swollen out in a balloon-shaped fashion and acquired slightly thickened walls. $\times 375$.

Fig. 8. Transverse section of an adult ascophore for comparison with Fig. 1. The net-like arrangement of the 'veins' is due to the number of carbonized conceptacle walls pressed together. $\times 25$.

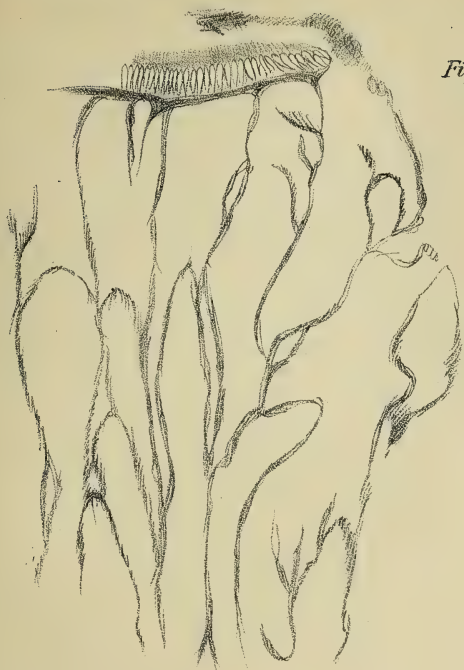


Fig. 1.

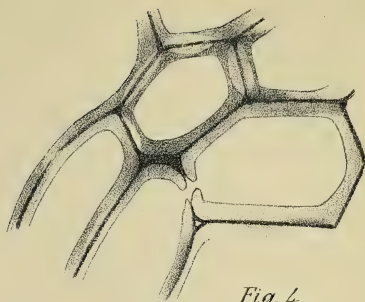


Fig. 4.

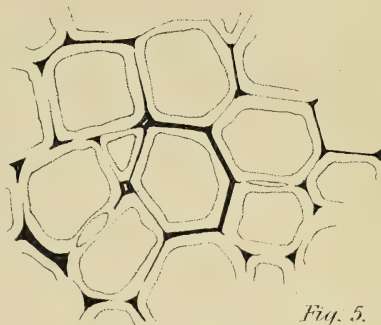


Fig. 5.

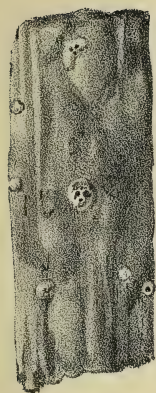


Fig. 2.

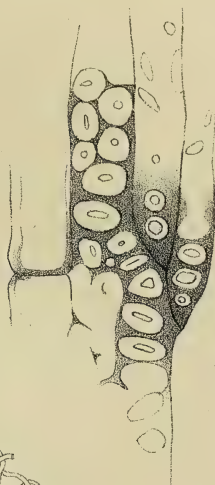


Fig. 3.



Fig. 8.

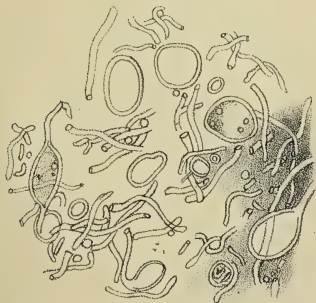


Fig. 7.

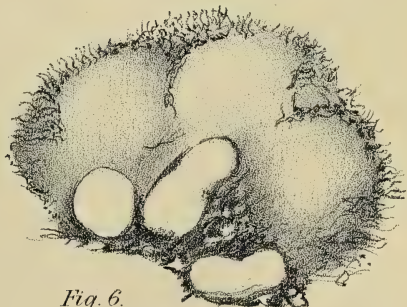


Fig. 6.

R.H.B. del.

University Press Oxford.

Infranodal Organs in Calamites and Dicotyledons.

BY

EDWARD C. JEFFREY, Ph.D.



With Plates VIII and IX.



I.

IN a recently published article¹ the writer has described his investigation of the development and anatomy of the genus *Equisetum*, and made some suggestions in regard to corresponding features in the structure of Calamites. During the past summer he has had the opportunity of examining several of the more important European collections of Calamitean fossils, and has been able to put to the test the suggestions made as a result of the study of the anatomy of *Equisetum*.

At the end of the article referred to above, appeared the customary summary of results and conclusions. In the fifth paragraph of the summary the statement is made: 'The branches of Calamites did not, as has been stated in recent years, arise above the nodes, but, like those of the Equisetaceae, originated either more or less exactly from the centre of the ring of nodal wood, or from its lower border.' In the sentence quoted the writer used the term node in the sense

¹ Mem. Bost. Soc. Nat. Hist., vol. v, No. 5.

in which it is ordinarily employed in the literature referring to the Equisetales, viz. as equivalent to the so-called nodal wood¹. In the memoir of Williamson and Scott² on Calamites, the term node is used in the ordinary botanical sense, viz. as marked by the exit of the leaf-traces. This different use of the term is the less apparent because these authors continue to employ the expression, 'nodal wood,' for the peculiar tracheary zone just above the insertion of the leaf-traces (i. e. node in their sense). It would seem to be preferable to abandon this term altogether and to designate the tracheary zone the supranodal wood, especially since the branches which they describe as originating 'above the node' are directly attached to the peculiar zone in question. It will be apparent from the above statement that, in regard to the mode of origin of branches in Equiseta and Calamites, there is no real difference of opinion between the writer and the authors cited above.

In the sixth paragraph of the conclusions the statement is made that 'the more conspicuous series of nodules on the medullary casts of Calamites are impressions not of Williamson's infranodal canals, but, on the contrary, of the short, cylindrical medullary cavities of modified rhizophorous branches, homologous with those of living Equiseta.' This statement rested on a number of data.

In the first place, the writer has called attention to the fact that in *Equisetum* the leaf-traces lie above the woody cylinder of the branches in the cortex of the main axis³, although they are originally below them⁴. In nearly all the figures which represent the stems of Calamites with the surface preserved (*Calamitina* of Weiss), the leaf-scars are placed below the branches. The writer argued, since 'the Calamite, so far as anatomy goes, is simply an *Equisetum* with secondary thickening⁵,' it was extremely improbable

¹ Cf. Seward, Fossil Plants, 1898, p. 251, and especially description of figure on p. 252.

² Phil. Trans. Roy. Soc., vol. clxxxv (1894), B. ³ Op. cit., Plate 29, Fig. 2.

⁴ Op. cit., Plate 26, Fig. 16.

⁵ Scott, Studies in Fossil Botany, 1900, p. 23.

that the surface of the Calamitean stem, properly orientated, should show the leaf-traces below the branches, since the opposite is the case in *Equisetum*. This view is strengthened by the fact that Weiss¹, in order to make the leaf-traces come below the branches, actually in one instance figures the scars of roots immediately above those of the branches to which they are related, whereas in *Equisetum*, as is well known, the roots always originate from the lower side of the branches. In another figure, which shows the leaves still attached to the surface of a fragment of Calamitean stem, and in regard to which, consequently, there can be no doubt as to the proper orientation, Weiss², in order to keep the leaves below the branches, is compelled to intercalate a node. In order to effect this, he interprets small rows of scars which appear along the lower margin of the cicatrices of the branches as belonging to a node in this position. The writer regards the small scars in question as representing the ochreolae or basal sheaths of leaves belonging to the branches left attached to the main stem after the removal of the latter, exactly as is the case in *Equisetum*. This view of the matter seems to be strengthened by the fact that Weiss' 'node' does not extend completely across the figure, but ends below the branch on the extreme right. The same feature is seen less clearly on the left. It has been recently shown³ that the bases of the leaves in some Calamites were fused to form a sheath, exactly as in the Equiseta of the present day. There is thus an additional reason for emphasizing the correspondence in the relation of branches and leaf-traces at the surface of the stem in the two groups. As a result of his examination of the fossils, the writer sees no reason to abandon the suggestions made in 1899, viz. the figures that represent the surface of the stem in Calamites are not unfrequently inverted or misinterpreted. This is, for example, specially true of those appearing in the beautiful memoirs of Weiss.

¹ Steinkohlen-Calamarien, Heft 1, p. 121.

² Ibid., Heft 2, Atlas, Plate 16, Fig. 6.

³ Scott, Studies in Fossil Botany, p. 35, Fig. 11.

Since there was, apparently, good reason for inverting the figures of the surface of Calamitean stems, the question arose whether a similar procedure should not be adopted in the case of those representing the course of the branches and leaf-traces through the secondary wood. In *Equisetum* the leaf-traces pass very quickly above the branches on their upward course in the cortex. Assuming that the disposition of the leaf-traces in *Equisetum* represents the course of the leaf-traces in the *young* Calamite, the writer was led to the conclusion that the leaf-traces should lie above the branches in the secondary wood of Calamites, since in modern plants, even where the leaves persist for several years, the course of their traces is practically that which is present in the young stem. Compare Photographs 1 and 9, Pls. VIII and IX. This view of the matter seemed to be supported by figures of Williamson reproduced in my memoir already mentioned. In these figures certain structures, imbedded for the most part in the so-called nodal wood, above the infranodal strands, are interpreted by Williamson as the beginnings of branches. Williamson and Scott subsequently announced that these were not branches, but leaf-traces. In *Equisetum* foliar traces are never found imbedded in the nodal wood, and branches do not occur over infranodal strands. By inverting these figures it became possible to regard these indications as belonging to branches. An examination of the fossils, however, has shown that the structures in question are really leaf-traces and not branches. Dr. Scott¹ has recently published an admirable figure of a tangential section of the wood of a Calamite, in which the indications are clearly recognizable as leaf-traces, which is not the case in all the earlier figures. This occurrence of the leaf-traces apparently imbedded in the so-called nodal wood, even in sections quite close to the medulla, is probably to be explained by the reduction of the metaxylem, since a similar reduction has been shown by Dr. Scott to be present in the internodal strands. It follows, of course, if the structures figured by Williamson in

¹ Studies in Fossil Botany, p. 25.

the nodal region of tangential sections of Calamitean wood, and at first interpreted by him as branches, are in reality leaf-traces, that the figures mentioned above are correctly orientated in their original position.

Another apparent reason for inverting the figures of tangential sections through the wood of *Calamites* was derived from the statement made by M. Renault, that certain organs occurring below the leaf-traces in the secondary wood bore roots. These organs he called 'organes rhizifères,' and identified them with Williamson's infranodal canals. If they were rhizophorous, it appeared to the writer that they could not well be other than reduced radiciferous branches, homologous with those of living *Equiseta*. In the *Equiseta* both the normal branches and the rhizophorous ones have the same relation to the leaf-traces, and the conclusion seemed warranted that the leaf-traces should lie above the normal branches of *Calamites* in the secondary wood, as well as above the organs described by M. Renault as rhizophorous.

Obviously, the argument in the last paragraph turns on whether the 'organes rhizifères' of M. Renault (infranodal canals of Williamson) were really radiciferous or not. Through the kindness of Dr. D. H. Scott the writer has had the opportunity of studying two beautiful series of tangential sections through bases of Calamitean branches bearing roots. Such specimens are extremely rare. An examination of the series showed that the roots were not attached to the infranodal organs, although the latter were present in abundance. The writer owes to the courtesy of M. Renault the opportunity of examining the Calamitean sections in the collections of the Jardin des Plantes. Although some of the preparations showed roots imbedded in the secondary wood, the writer was unable to discover that in any case they were related to infranodal organs. M. Renault's statement that roots were attached to the infranodal organs possibly depends on the observation of Weiss, that the infranodal tubercles are absent on the medullary casts of aërial portions of *Calamites*. Grand'Eury also, in one of his older articles on *Calamites*,

notes that the infranodal tubercles occurred on the medullary casts in subterranean parts of *Calamites*, and gradually disappeared as the stem passed above the surface of the soil. The subterranean position of the infranodal organs probably suggested to M. Renault their relation to roots. In a recent account¹ of the casts of *Calamites*, Grand'Eury has described more fully the distribution of the infranodal tubercles. He states that they are absent on the horizontal rhizomes, although these are abundantly provided with roots, and that they occur only on the ascending portions of the subterranean stems of *Calamites*. It is apparent from these recent observations that there is no necessary relation between the presence of roots and the occurrence of infranodal tubercles. It follows from the various data introduced in this paragraph that the infranodal organs of *Calamites* were not rhizophorous, and further, that, as a consequence, they were not radicleferous branches. Hence they do not supply an argument for the inversion of figures representing tangential sections through the secondary wood of *Calamites*.

Convinced by the study of the fossils that the infranodal organs of *Williamson* were not to be explained as the equivalents of the rhizophoric branches of the *Equiseta*, the writer has still felt that it was improbable that they should be without analogues in living plants. He has devoted his attention particularly to marsh-plants with fistular medullary cavities separated by diaphragms, with a large number of leaf-traces passing off in the region of the nodes, and with considerable secondary wood. The result has been the discovery of structures which appear to differ in no important respect from the infranodal canals (lenticular organs) of *Williamson*. Some account of these is given in the following paragraphs.

II.

Potentilla palustris is a plant which is particularly characteristic in respect to infranodal organs. Photograph 3, Pl. VIII,

¹ Forêt Fossile de *Calamites* Suckowii, Comptes Rendus, 1897.

shows the structure of the woody cylinder of a biennial stem of this species a little below the region of a medullary diaphragm. It is evident that the continuity of the woody zone is interrupted at approximately equal intervals by broad parenchymatous bands running from the persistent zone of medullary tissue just inside the fibrovascular cylinder to the cortex. Photograph 4, Pl. VIII, represents one of these parenchymatous organs more highly magnified. Certain dark-walled elements may be distinguished among the parenchymatous cells; these are moribund cells corresponding to those of the fistular portions of the pith. In Photograph 5, Pl. VIII, is to be seen a radial section through one of these parenchymatous organs. The zone of secondary wood maintains its full thickness above the organ, while below it becomes narrower in passing upwards. On the right above is the medullary diaphragm. Photograph 6, Pl. VIII, shows the parenchymatous organ more highly magnified. Externally is to be seen a layer of periderm. Certain of the cells of the parenchymatous organ are dead, and show dark in the photograph. On the lower side of the parenchymatous organ a leaf-trace may be seen passing upwards and outwards. Photograph 7, Pl. IX, is of a tangential longitudinal section of the nodal region of the woody cylinder. Two parenchymatous organs are to be seen, and at their lower margins may be made out two corresponding leaf-traces in oblique transverse section. The parenchymatous interruptions of the woody cylinder illustrated in the photograph described above occur always opposite the leaf-traces and a little below the medullary diaphragm. They cause the only breaks in the continuity of the woody zone, and are, as the writer will attempt to show subsequently, to be regarded as the upper ends of the foliar lacunae which have not been closed up by the formation of secondary wood.

Cicuta maculata is another marsh-plant with numerous leaf-traces passing out in the nodal region, and with a considerable secondary growth of the ligneous zone. Photograph 8, Pl. IX, represents the nodal region of a woody

cylinder which has been deprived of its cortex and phloem by maceration. The cylinder has been split and the two halves placed side by side. A row of lenticular cavities may be seen crossing the axis, each of which subtends a leaf-trace. In Photograph 9, Pl. IX, is represented a radial section passing through one of the parenchymatous organs. Above, on the left, is the medullary diaphragm. Passing obliquely outwards is a leaf-trace which originates from the lower node and passes upwards, opposite the secondary wood, to make its exit just below the higher node. This photograph shows clearly that the parenchymatous organs under discussion are really the upper ends of the foliar lacunae, and at the same time are infranodal.

Photograph 10, Pl. IX, is of a transverse section of the stem of *Cornus stolonifera* at the height of exit of the leaf-traces. The parenchymatous gaps are six in number in this case, three for each leaf. The lacunae are here very small in size, as is usually the case with plants with more slender stems and denser texture than those described in former paragraphs. In Photograph 10, Pl. IX, the same stem is shown in transverse section at a region considerably below the node, and indentations may be seen in the woody zone corresponding to the leaf-traces. Photograph 12, Pl. IX, shows the same stem at a point just above the region where the woody cylinder is interrupted by the exit into the cortex of the leaf-traces of the next lower phytomere. The foliar indentations in the woody cylinder have disappeared. This section is to be regarded as passing through the nodal region; for just above it the leaf-traces begin their upward and outward course in the secondary wood, which is only completed immediately below the next node. The gaps in the woody cylinder in this case also correspond to the outgoing leaf-traces, and are likewise below the node.

The writer has made similar observations in a number of other cases, and it appears to be not uncommon in plants possessing secondary growth that the leaf-traces should pass upwards in the secondary wood, and outwards into the cortex

near a higher node. On the other hand, in some instances the foliar strands may pass to the higher node in the cortex, e.g. *Menyanthes trifoliata*, &c. It seems unnecessary to go further into this matter at the present time. It is worth while, however, to emphasize in this connexion that the only criterion of the node in plants with secondary growth is the point of exit of the leaf-traces from the primary wood.

The interesting feature of these observations seems to be that parenchymatous organs, lenticular in shape and infranodal in position, occur in a number of Dicotyledons with secondary growth. Where the leaf-traces are verticillate and medullary diaphragms are present, these organs resemble most strikingly the infranodal organs of the *Calamites*. If a very large stem, possessing the structure of that of *Potentilla palustris*, were to have its parenchymatous parts macerated away and its medullary cavity filled with mud, the latter would produce a cast not unlike the very characteristic casts of *Calamites*. The only essential difference would be the fact that the constrictions in this case would really be infranodal, and not represent rings of tracheary tissue above the nodes, as in *Calamites*.

The infranodal organs of certain palustrine Dicotyledons differ morphologically from those of the *Calamites* in the fact that they are the upper ends of foliar lacunae of the woody cylinder, whereas those of *Calamites*, as the writer has attempted to show, are the upper open ends of ramular lacunae. This is an important difference from the phylogenetic standpoint, since it unites the Angiosperms with Filicinean ancestors, whilst for the *Calamites* a Lycopodiaceous ancestry is indicated. It is interesting that analogous structures should appear in groups genetically so remote, under similar conditions in life, and affords a striking example of the efficiency of homoplasy in originating similar structures.

In conclusion, a few words should be said on the relation of branches and roots to the lenticular organs, although it is not necessary to go into this matter at length, since Dr. Scott will doubtless deal with it sufficiently in his

description of the newly discovered root-bearing stems of Calamites in his possession.

In *Equisetum*, as is well known, the branches occur immediately above the medullary rays, and since the roots in this genus are always attached to the bases of the branches, they consequently occur in the same position. In *Equisetum hiemale* it not unfrequently happens, in the case of deep and vigorous rhizomes, that the rhizophorous branch almost completely aborts, and the single very large root, which it bears, is attached directly to the supranodal wood, thus lying immediately over the large ramular medullary ray. What occurs exceptionally among the Equiseta seems to have been the rule in the case of the Calamites; for in the latter it is quite unusual to find a branch with roots attached. Weiss figures only one example of this sort.

Photograph 2, Pl. VIII, represents a tangential section through the wood of *Polygonum amphibium* in the nodal region. Above is to be seen the parenchymatous medulla of a branch, and immediately below the latter is a lenticular organ with a leaf-trace causing a toothed projection on its inferior margin. In nearly the same horizontal plane on the left is another lenticular organ (infranodal canal). In the lower part of the photograph a root is seen in cross-section. So far as the writer has been able to observe, the roots of the Dicotyledons have no fixed position in regard to either the nodes or the branches. The relation of the branch to the infranodal organ in the Dicotyledons exactly simulates the relation of the roots to the similar organs of the Calamites; for in the latter the roots occupied the same position as branches just as they do exceptionally in *Equisetum hiemale*.

The results of the present investigation may be summarized as follows:—

1. Some of the existing figures of the surface of Calamitean stems are inverted and others are misinterpreted.
2. The infranodal organs of Williamson were not rhizophorous.

3. The structures figured by Williamson apparently imbedded in the supranodal wood of *Calamites* are not branches, as he originally stated, but, on the contrary, are leaf-traces, as subsequently suggested by Williamson and Scott.

4. As a consequence of paragraphs 2 and 3, and other minor considerations, the writer's earlier suggestion that the figures of tangential sections of the secondary wood of *Calamites* should be inverted is not to be accepted.

5. Analogues of Williamson's lenticular organs (infranodal canals) exist in certain palustrine Dicotyledons of the present day.

The writer is under great obligations to Sir William Thiselton-Dyer and Dr. D. H. Scott for the hospitality of the Jodrell Laboratory at Kew, and to the latter for free access to his collection of fossil plants. Thanks are also due to Dr. Henry Woodward and Mr. Newton of the Museum of Natural History, South Kensington, for the opportunity of examining the large series of palaeobotanical sections in their charge. Last, but not least, he wishes to express his gratitude to M. Renault, of the Museum at the Jardin des Plantes, for very kindly permitting him to examine his preparations illustrative of Calamitean anatomy.

DESCRIPTION OF THE PHOTOGRAPHS IN
PLATES VIII AND IX.

Illustrating Dr. Jeffrey's paper on Infranodal Organs.

PLATE VIII.

Photograph 1. Radial section through the wood of *Abies balsamea*, showing the course of a leaf-trace. $\times 30$.

Photograph 2. Tangential section of the subterranean stem of *Polygonum amphibium*.

Photograph 3. Transverse section through the infranodal region of the stem of *Potentilla palustris*; the woody cylinder is interrupted by parenchymatous stripes. $\times 8$.

Photograph 4. The same, showing one of the parenchymatous stripes more highly magnified. $\times 40$.

Photograph 5. Radial section through a parenchymatous stripe of the same species, which shows that it is situated below the medullary diaphragm. $\times 10$.

Photograph 6. Radial section showing the parenchymatous stripe more highly magnified. $\times 40$.

PLATE IX.

Photograph 7. Tangential section through the wood of *Potentilla palustris*, showing two parenchymatous stripes (infranodal organs). $\times 10$.

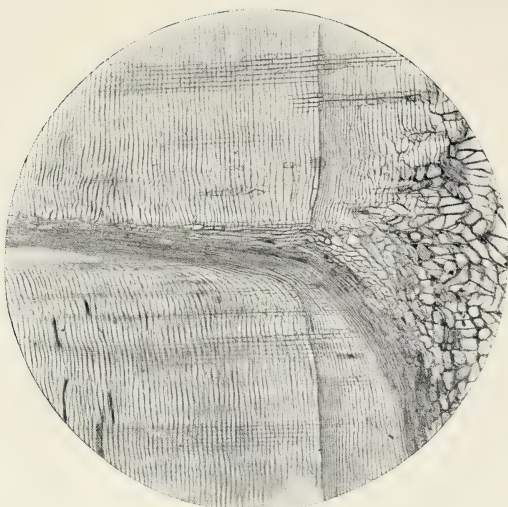
Photograph 8. Superficial view of a split stem of *Cicuta maculata*, showing the infranodal lenticular organs; the cortex and the bast have been macerated away. $\times 2$.

Photograph 9. Radial section passing through two nodes of the same species, showing the oblique course of the foliar bundle. $\times 7$.

Photograph 10. Transverse section through the region of exit of the foliar traces in *Cornus stolonifera*. $\times 10$.

Photograph 11. Transverse section of the same, some distance below the node, showing the indentations in the secondary wood caused by the leaf-traces. $\times 10$.

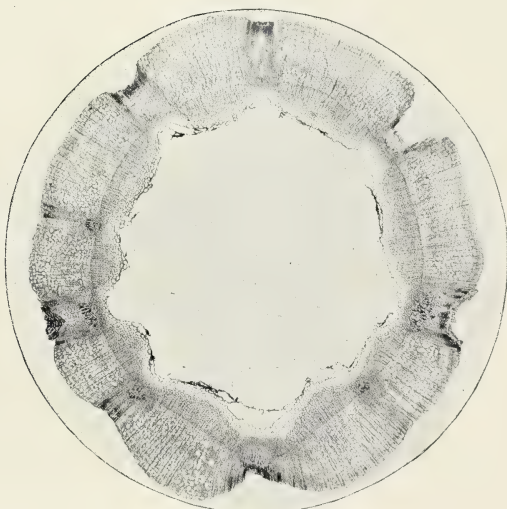
Photograph 12. Transverse section through the nodal region of the same species; the indentations have disappeared. $\times 10$.



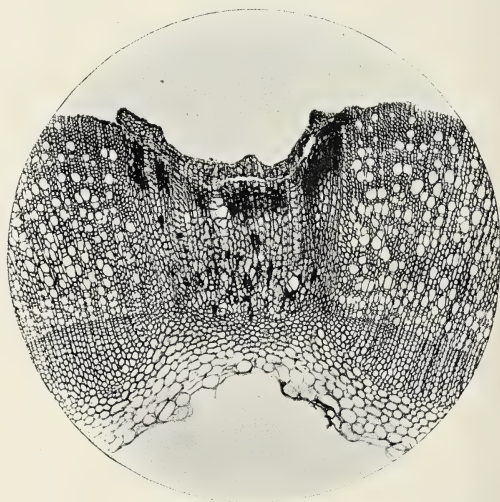
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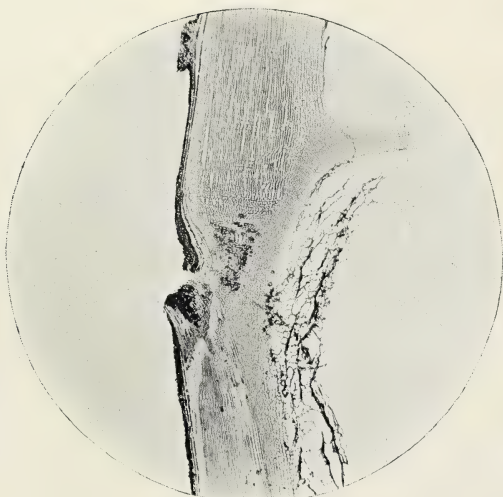
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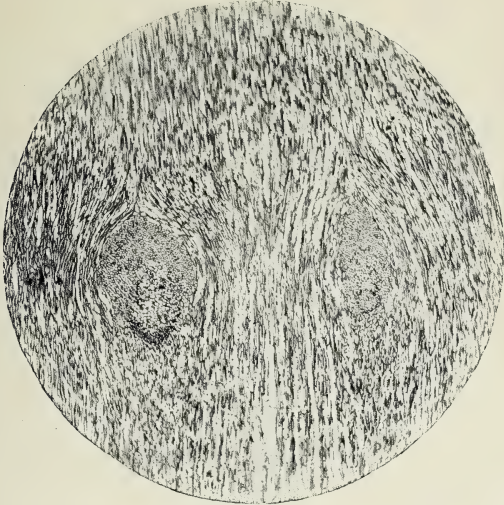
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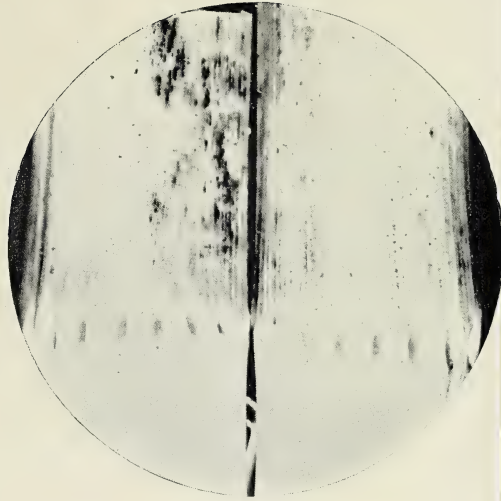
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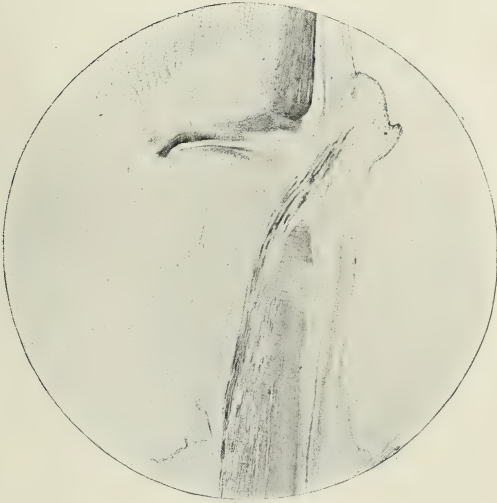
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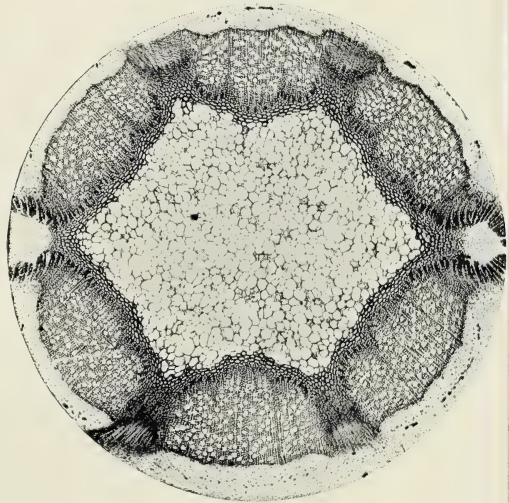
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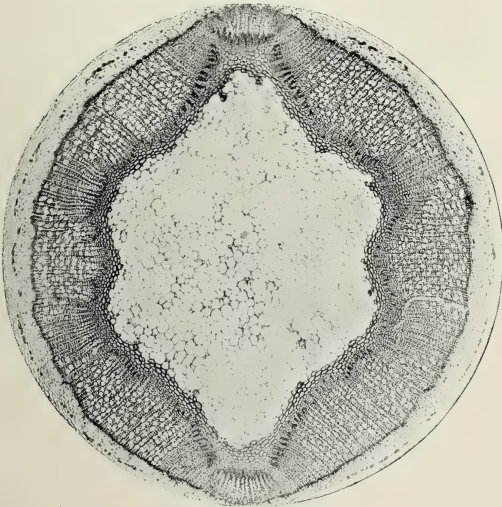
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Nuclear Studies on Pellia¹.

BY

BRADLEY MOORE DAVIS.

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With Plates X and XI.
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THE Hepaticae present an exceedingly attractive field for research to the student of the plant-cell. There is much of interest in the special peculiarities exhibited in the arrangement and organization of the elements in the sporogenous and vegetative tissues and in the details of mitoses, which have many distinctive features. However, to the writer's mind, the Liverworts are most interesting in this connexion because they offer the possibility of solving certain problems of great importance to our understanding of the morphology of the plant-cell, and particularly the conditions characteristic of nuclear divisions in the higher plants, Spermatophytes and Pteridophytes.

Since 1897 a number of contributions have appeared, dealing with karyokinesis in many types from these two groups, and considering a variety of tissues both reproductive and vegetative². Naturally the most striking results have

¹ Contributions from the Hull Botanical Laboratory, No. 25.

² The papers have considered such a variety of forms that a brief list of the most important contributions is worth a moment's notice. Arranged approximately in order of publication we have Osterhout ('97) on *Equisetum*; Mottier

[Annals of Botany, Vol. XV. No. LVII. March, 1901.]

come from studies upon the spore-mother-cell, pollen-mother-cell, and the embryo-sac. But more recently several detailed investigations have treated of vegetative tissue. It is not necessary to review this literature at this time. The discussion of the centrosome problem is fresh in the minds of all interested in plant-cytology, and, besides, the subject has recently been treated in great fullness by Strasburger ('99). In spite of contradictory reports, the writer feels that there is sufficient accord among investigators to justify some general statements of certain events of mitosis.

It appears probable for the Spermatophytes and Pteridophytes that the spindles in the spore-mother-cell and its homologues arise from fibrillae developed in the kinoplasm around the nucleus. In early stages of mitosis the fibrillae may radiate from the nucleus, and later converge into brush-like groups. Or the fibrillae may surround the nucleus as a closely packed felt with a general parallelism of direction; such that an axis may be early recognized. When the fibrillae are in brush-like bundles they sooner or later arrange themselves to form two general groups, that come to lie on opposite sides of the nucleus, and determine the poles of the spindle.

The behaviour of kinoplasm in vegetative tissue at the time of mitosis is more difficult to follow, but in the examples best known from the studies of Hof ('98) and Nemec ('99) the spindle-fibres are preceded by accumulations of kinoplasm

('97 and '98) on *Lilium*, *Podophyllum*, and *Helleborus*; Juel ('97) on *Hemerocallis*; Ishikawa ('97) on *Allium*; Sargent ('96 and '97) on *Lilium*; Calkins ('97) on *Pteris* and *Adiantum*; Guignard ('97) on *Nymphaea*, *Nuphar*, *Limodorum*, and *Magnolia*; Coulter, Chamberlain, and Schaffner ('97) on *Lilium*; Lawson ('98) on *Cobaea*; W. C. Stevens ('98*a* and '98*b*) on *Scolopendrium* and *Asclepias*; Schaffner ('98) on *Allium*; Fulmer ('98) on *Pinus*; Cavara ('98) studies chiefly upon nucleoli and chromatin in a variety of forms; Hof ('98) on *Pteris*, *Aspidium*, *Ephedra*, and *Vicia*; Belajeff ('97 and '98) on chromosome-reductions in several forms; Blackman ('98) on *Pinus sylvestris*; Chamberlain ('98) on *Pinus Laricio*; Nemec ('99*a* and '99*b*) on *Allium* and *Solanum*; Atkinson ('99) on *Arisaema* and *Trillium*; Duggar ('99) on *Bignonia*; Wiegand ('99) on *Convallaria* and *Potamogeton*; Grégoire ('99) on *Lilium* and *Fritillaria*; Guignard ('99) on *Najas*; Strasburger ('99) on *Tradescantia*, *Iris*, *Larix*, *Osmunda*, *Nymphaea*, *Lilium*, and other types; Williams ('99) on *Passiflora*; Duggar ('00) on *Symplocarpus* and *Peltandra*; Lawson ('00) on *Gladiolus*; Smith ('00) on *Osmunda*.

on opposite sides of the nucleus, forming two caps extending over the ends of that structure. The kinoplasm composing the caps is at first granular, but finally the greater part of the substance is changed into fibrillae.

It has not been possible to establish centrosomes either giving rise to or accompanying the activities of the kinoplasm, and the close study of the development of the spindle, together with the absence of the structure known as the aster, has led to a retreat from the views very generally held previous to 1896 respecting the presence of centrosomes in the cells of Pteridophytes and Spermatophytes. It is probably the belief of most plant-cytologists that neither the centrosome nor the aster is present in the cells of vegetative tissue in these plants or in that phase of ontogeny when the sporophyte passes over to the gametophyte.

The most interesting problem in spindle-formation, apart from the very difficult physiological explanation of the behaviour of fibrillae, is perhaps the question of phylogenetic evolution. How is the condition in the Pteridophytes and Spermatophytes, where the fibrillae are largely free and distinct from one another and acting independently of a fixed centre, to be related to centrosomes and asters present in certain Thallophytes? The problem is one of very broad interest, as it involves a form of protoplasmic differentiation apparently restricted to the higher plants, and not found in the animal kingdom.

Investigations among the Thallophytes have proceeded sufficiently far to establish the presence of beautiful asters for the several groups, in certain instances with definite centrosomes. The most notable examples are presented by the Phaeophyceae in *Fucus* (Farmer and Williams, '96 and '98, Strasburger, '97); *Sphacelaria*, studied with the greatest detail by Swingle ('97), and *Dictyota* (Mottier, '98 and '00). From the accounts of Lauterborn, '93 and '98, we may expect these structures in the Diatoms, but other groups of Algae are less promising, although they have been very little studied. *Corallina*, a member of the Rhodophyceae (Davis, '98), has

remarkable centrosphere-like aggregations of kinoplasm at the poles of the spindles in the tetrasporangium, but these structures are not permanently present in the protoplasm. We know almost nothing of spindle-formation in the Chlorophyceae, but observations on the development of zoospores indicate that kinoplasm may there take form and appear as blepharoplast-like bodies (Strasburger, '99). The conditions in the Fungi of course only indirectly affect the problem of phylogeny concerned with the Hepaticae. However, especially well-defined centrospheres and conspicuous asters occur in the ascus (Harper, '97), and centrosomes have been reported in several other groups.

One more class of plants, the Bryophytes, remains for our consideration in this brief statement of our present knowledge respecting the aster. Of spindle-formation in the Musci nothing has been published, and the members of this group are certainly not promising subjects for study, the cells and nuclei being relatively small. But among the Hepaticae we meet with forms whose cells are admirably adapted to a minute examination. Farmer ('94 and '95) studied a number of genera, reporting conditions of great interest. He described asters and centrosomes (Farmer and Reeves, '94) for the early mitoses in the spores of *Pellia* as they germinate in the sporangium, and his results were confirmed by Strasburger ('95) from Farmer's own preparation. Schottländer ('92), before Farmer, had noted centrosomes in the sperm-mother-cells of *Marchantia*, but without attendant radiations. His conclusions, however, are open to criticism on account of the methods employed, and further study of this point is much to be desired.

Of greater interest than the aster in *Pellia* is Farmer's account ('94) of a quadripolar spindle in the spore-mother-cell of *Pallavicinia*. This unique condition is said to arise from a special investing zone of 'archoplasm' around the nucleus. The kinoplasmic material extends into the four lobes of the spore-mother-cell, forming a four-rayed star with the nucleus in the centre, each ray becoming a pole of the spindle. The

chromosomes, four in number, are organized in the nucleus, and by a doubling division are increased to sixteen as mitosis proceeds. Four chromosomes then travel along each division of the four-poled spindle. Finally the four rays of the star-like figure break apart at the centre, the kinoplasm ('archoplasm') in each contracting, with its group of chromosomes, and shortly afterwards the four daughter-nuclei are organized.

In a note and an extensive paper which appeared in the following year Farmer ('95*a* and '96*b*) considers a number of species of Hepaticae distributed through several genera. With respect to the quadripolar spindle he did not find it very generally present. There are two successive divisions closely following one another in the spore-mother-cells of the Marchantiales (*Fegatella*, *Fimbriaria*, and *Plagiochasma*) and the Ricciales. Each mitosis is entirely distinct, but in some instances the first presented the arrangement of the chromosomes known as heterotype. Among the Jungermanniales there was some diversity. *Pallavicinia*, *Fossombronina*, *Pellia*, *Scapania*, *Cephalozia*, *Lophocolea*, *Frullania*, and *Aneura* have, according to Farmer, quadripolar spindles at least in the prophase of mitosis, but sometimes bipolar spindles result from the apparent coalescence of the four original centres. In *Riella* there is never any indication of a quadripolar spindle, but instead, two mitoses following one another, with spindles of the usual bipolar type. However, the latter condition, identical with the usual order of mitoses in the spore-mother-cell, is believed by Farmer to have arisen from the four-poled spindle. He considers certain species of *Pellia*, *Aneura*, *Scapania*, and *Cephalozia* as presenting transitional stages in the process whereby the four centres of the quadripolar spindle, fusing in pairs, form a spindle of the bipolar type.

The quadripolar spindle is then regarded by Farmer as well established for the spore-mother-cell of *Pallavicinia*, and as present but not permanent in the prophase of the homologous mitoses in many other Jungermanniales. In the latter forms it is replaced by the two successive mitoses almost universally present in spore-mother-cells, but Farmer believes these to

have been derived from the quadripolar spindle through the convergence and fusion of the four centres in pairs. Farmer also believes that the quadripolar spindle is associated with and conditioned by the peculiar character of the four-lobed spore-mother-cell. These are very fundamental and interesting suggestions whose establishment would be important contributions to plant-cytology.

A quadripolar spindle, such as Farmer has described for *Pallavicinia*, whose four centres act simultaneously so that the chromosomes separate into four groups, is quite unknown in other plants or in animals. It should be investigated with great care, for upon the fact may depend much of theoretical interest in the problem of the evolution of mitotic phenomena in the spore-mother-cell. If the quadripolar spindle merely accompanies the curious four-lobed spore-mother-cell of the *Jungermanniales* it may be entirely without morphological significance. But the theoretical considerations involved take on new interest from the facts of double longitudinal splitting of the chromosomes during the first mitosis in the spore-mother-cell, described for a number of forms by Guignard ('99) (*Naias*) and Strasburger ('99).

The writer has not been able to examine much material of *Pallavicinia*, and wishes only to express his fear that Farmer may have misinterpreted his preparations. As will appear from the present investigation of *Pellia*, as well as the examination of *Anthoceros* (Davis '99), the writer holds views on the method of spindle-formation in the *Hepaticae* very different from those of Farmer. But results in studies of this character come so slowly that one may well hesitate to advance a theory covering an entire group. However, after the account of this investigation, the reader will find some suggestions in that portion of the paper headed 'General Considerations.'

The material, *Pellia epiphylla*, was collected during the summer and autumn of 1899 from several localities, but chiefly from rocky gorges near Starved Rock, Illinois. The mitoses of the spore-mother-cell take place early in October so that the spores are fully developed before frost. Indeed

the earlier nuclear divisions of the gametophyte phase occur in the spore before winter, so that the spore in the following spring is multinucleate. Therefore material collected during October is almost sure to furnish a considerable variety of stages. There is a period of several days when the spore-mother-cells are, one may say, ripe for division, and such plants brought from the cold rocks to a warm room will immediately develop spores. Thus in a relatively short time complete sets of stages may easily be obtained.

A number of fixing agents were employed, but 1 % chrom-acetic acid and the weak formula of Flemming proved to be the most satisfactory. In this case there seems to be little choice between the two killing fluids when one understands certain peculiarities of the safranin and gentian violet stains. These stains hold more readily in Flemming-fixed material, but the manipulation may be regulated to give very clear results after chrom-acetic acid. Material fixed in corrosive sublimate and picro-sulphuric acid proved thoroughly unsatisfactory, that in absolute alcohol, sublimate-acetic and Carnoy's fluid was somewhat better, and Hermann's and von Rath's formulae approached most nearly the excellence of weak Flemming and chrom-acetic acid. The sections were stained on the slide with safranin and gentian violet or with haematoxylin after the method of Heidenhain.

Mitosis was studied at three periods of ontogeny, viz., in the seta of young sporophytes, in the spore-mother-cell and at the beginning of the gametophyte period in the germinating spores. It is most convenient to begin our account with the events of sporogenesis, as the spore-mother-cell so generally presents cytological phenomena most favourably.

The spore-mother-cells of *Pellia* are conspicuously four-lobed, a fact that seems to be generally true of the Jungermanniales. This character appears several weeks before the spores are formed. The spherical cells of the archesporium come to lie freely in a mucilaginous matrix, and develop these lobes simultaneously and symmetrically in four divergent directions. The spherical nucleus lies in one of the lobes

until shortly before the time of sporogenesis, when it may be found, irregular in outline, in positions nearer to the centre of the cell (see Pl. X, Fig. 1), and evidently being carried to that situation by movements of the cytoplasm. The evidences in fixed material of such cytoplasmic activity are dense areas, strands and stream-like structures such as are found in cells where similar activities are known to occur. The nucleus is mobile, changing its form when necessary as it is carried to its final resting-place in the geometrical centre of the cell. As is shown in Figs. 2 and 3, the nucleus almost completely fills the space at the point of junction of the four lobes.

There now follows a period of rest for several days, during which two events happen. Previous to this time the linin network has not been very conspicuous in the nucleus, but it now begins to stain deeply. At this time synapsis occurs in a marked and unmistakable form (see Fig. 3).

Farmer recognized and described the event in detail. The linin material gathers in a close granular tangle at the side of the nucleolus, and all the rest of the interior is quite free from chromatin. Synapsis appears with perfect regularity at this period of ontogeny, agreeing with the writer's observations on *Anthoceros* ('99, p. 97) and the general results of studies in sporogenesis for the higher plants. There is no more reason for regarding the phenomenon as an artefact than in *Anthoceros*, where the conditions for a comparative study of the effects of fixing fluids on the cells were most favourable.

The nucleus, on emerging from synapsis, presents a delicate, closely wound spirem-thread with a single row of deeply staining granules imbedded in the linin material. The segmentation of the spirem-thread and formation of the chromosomes proceed immediately, but we shall defer for the present the discussion of those phenomena.

With the spirem-condition, as is shown in Fig. 4, we find the nucleus ready to enter the early prophase of the first mitosis. The nucleolus becomes vacuolate, and finally frag-

ments and dissolves, but not until the chromosomes are organized and mitosis has nearly reached the metaphase. The changes that most interest us are, however, those of the kinoplasm. The homogeneous granular zone becomes differentiated by irregular lines plainly made up of rows of granules. These soon take the definite form of fibrillae distributed irregularly around the nuclear membrane. But the fibrillae develop most numerous in the four regions where the lobes of the cell diverge from the centrally placed nucleus, as is shown in Fig. 5. Farmer reported the presence of four centrosomes at the points corresponding with the lobes of the spore-mother-cell, but I have failed to find such structures in my preparations.

The nuclear membrane becomes faint and then irregular in outline (Fig. 4), finally disappearing, whereupon the fibrillar kinoplasm immediately begins to occupy the nuclear space, previously clear except for the chromosomes, nucleoli and some granular material. Interesting stages in this process of dissolution are shown in Figs. 6 and 7. In the former case the fibrillae have begun to enter the nuclear space on two sides. In the latter we find the remnant of the nuclear membrane at one point, all the rest of the area being filled with delicate fibrillar kinoplasm.

It is at this period that we have appearances most similar to the quadripolar spindle reported by Farmer ('94) for *Pallavicinia*, and likewise described and figured for *Pellia*. The nucleus may become four-pointed and each point capped with fibrillar kinoplasm actually extending into the four lobes of the spore-mother-cell which are to become the spores. Fig. 5 shows such a condition previous to the dissolution of the nuclear membrane, and the appearance becomes far more marked later (see Figs. 6 and 7), when the kinoplasm increases in quantity. This condition is apparently the stage believed by Farmer to be related to a quadripolar spindle, but the writer cannot attach to it the same importance as that author.

It is true that preparations may be so deeply stained that the nucleus has the clear outline of a four-pointed star, but

such appearances give very little idea of the real structure. The four fibrillar cones are derived from and lie in the zone of kinoplasm which at this period is irregularly distributed, extending into the lobes of the spore-mother-cell. Although the fibrillae are most conspicuously massed in the cone-like bundles, they also completely envelop the nucleus, as may be plainly seen in sections that show a surface view (Fig. 8). The fibrillar cones have no bodies at their tips that might be considered centrosomes. On the contrary, they are generally sheathed by kinoplasm distinguished from the neighbouring cytoplasm by its freedom from granular matter and plastids. The larger number of fibrillae at these points may readily be explained by the accumulations of kinoplasm, which precede the development of the cones, and become evident before the fibrillae themselves appear.

Morphology, as far as the writer's studies are concerned, presents no evidence that the accumulations of kinoplasm and the accompanying cones of fibrillae are developed or controlled by any protoplasmic elements in the cells, such as centrospheres. The preparations of the spore-mother-cells have never presented such appearances as are found with centrospheres and asters. When first visible the fibrillae are distributed all around the nucleus; but shortly afterwards the cone-like bundles develop sometimes simultaneously, but not infrequently in such a manner that one or two are far more prominent than the others.

It is plain that the cones of fibrillae result from accumulations of kinoplasm that precede them in position around the nucleus. Why the kinoplasm should occupy so conspicuously the regions opening into the lobes of the spore-mother-cell seems obvious when we note the position of the nucleus. The nucleus, as may be seen from the figures, quite fills up the space common to the four lobes, and almost touches the cell-wall along the lines of constriction. The accumulations of kinoplasm most naturally then take positions of least resistance, and extend into the lobes of the spore-mother-cell. The four-rayed star is a transitory condition of prophase.

The cones of fibrillae are not spindles, and the figure presented is very far from the metaphase of mitosis. Farmer holds that the arms of the quadripolar body represent the astral portions of incomplete spindles, but he makes it plain that these arms in *Pellia* act in pairs, so that nuclear division is after the normal type, with two successive mitoses in place of a simultaneous division of the nuclear into four, as in *Pallavicinia*. It is a phenomenon of prophase, and the true spindle has a very different structure.

The mitoses in the spore-mother-cell are two in number and successive, with a short period of rest between the first and second division, during which the two daughter-nuclei pass into a resting condition. The spindle presented at the metaphase of the first mitosis is remarkable for the great breadth of the poles and the very large number and delicacy of the fibres that traverse its axis. Not infrequently one of the poles will be so broad that it actually forks (see Fig. 9), part of the fibres lying chiefly in one lobe and the remainder chiefly in another. In other cases the spindle will present the appearance shown in detail in Fig. 10 and characterized by the broad rounded poles. It should be noted that the delicate spindle fibres end in a region of protoplasm rather free from large granules, which is bordered by cytoplasm containing plastids and having a spongy structure. The writer's preparations gave no hint that these poles were ever occupied by bodies comparable to centrospheres or centrosomes. On the contrary the broad, flat, rounded or concave poles indicated that the spindle-fibres were not influenced by a well-defined centre. While the spindle is not multipolar in the manner so clearly shown in certain Spermatophytes, there being no conspicuous separate bundles of fibres, nevertheless the conditions are essentially similar. The parallel spindle-fibres are not gathered to a point but are distributed over a very broad area.

While the daughter-nuclei are being organized, the spindle-fibres grow fainter at the poles, and very shortly a line of granules appears in the equatorial zone (see Fig. 11) marking

the position of the cell-plate. The facts agree well with the theory recently advanced by Timberlake ('00) that kinoplasm is actually drawn to the cell-plate by the contraction and transformation of the spindle-fibres. According to this view Fig. 11 presents a spindle shortened to the form of a barrel between the daughter-nuclei. It is important to note that the cell-plate is laid down in *Pellia* after the usual manner in the Pteridophytes and Spermatophytes.

The writer's studies on *Anthoceros* (Davis '99, p. 103), led him to the conclusion that the cell-walls dividing the spore-mother-cell bore no relation to spindle-fibres, but were formed from films of protoplasm that developed between the pairs of nuclei and chloroplasts. The nuclear figures of the second mitosis in *Anthoceros* are so small that the difficulty in following the fate of the spindle-fibres was frankly acknowledged. They seem to entirely disappear before the cell-plate is laid down, the latter being first a delicate film held in place by numerous strands of cytoplasm. It is of course possible that this film is composed, at least in part, of material derived from the spindle-fibres, but such a relation could not be traced. The writer suggested that the film might be independent of the spindle-fibres and related to the plasmic membrane (Hautschicht). Perhaps more favourable species of *Anthoceros* may make clear the difficulty¹. It is certainly important that we should emphasize the fact that the process of cell-formation in *Pellia* agrees in all essentials with that found in the higher plants.

The daughter-nuclei following the first mitosis pass quickly into a condition in which the chromosomes break up into

¹ Since the above was written observations have been published upon a species of *Anthoceros* more favourable for study (Botanical Gazette, xxx. 395, 1900). Mr. J. M. Van Hook was fortunate in having material of an Italian form whose cells are larger than in our native species and yield more clearly the details of structure. He has established the presence of a small cell-plate between the daughter-nuclei after the second mitosis with the usual relation to connecting spindle-fibres. This cell-plate determines the position of the conspicuous film of protoplasm that later becomes transformed into the cell-wall. Thus *Anthoceros* presents no obstacle to extending through the Liverworts the general rule of cell-formation exemplified in higher plants.

fine granular material that becomes distributed over a linen network. Nucleoli are also developed, and the nucleus finally has every appearance of being completely in a state of rest (see Fig. 12). These facts are of some importance in view of the opinions generally held that the chromosomes maintain their individuality in the daughter-nuclei after the first mitosis of the pollen-mother-cell. This conception is intimately concerned with, and necessarily a part of, the recent studies of Guignard ('99) and Strasburger ('99), on the double longitudinal splitting of the mother-chromosomes. The appearance of the daughter-nuclei following the first mitosis in Spermatophytes is not suggestive of a resting nucleus, and yet it is very difficult to trace individual chromosomes in the irregular network of deeply staining chromatic material in the interior. However, some types are more favourable than others, and Guignard ('99) was fortunate in finding such an example in *Naias*. Strasburger has studied several forms (especially *Tradescantia*, *Larix*, *Allium*, and *Podophyllum*) in which the chromosomes may be followed with certainty through the period between the first and second mitosis. The peculiar difficulties of investigations upon this point have been due in part to the interesting double longitudinal splitting, by which the grand-daughter-chromosomes of the second mitosis are prematurely organized during the first. The daughter-nucleus, following the first mitosis, contains the grand-daughter-chromosomes usually grouped with some definiteness in pairs, and such pairs stand for the daughter-chromosomes of the first mitosis.

In *Pellia* we have a type where the chromosomes cannot be followed after the first mitosis, probably for the reason that they are relatively small. Whatever may be the significance of the deeply stained granules, one cannot discover any regularity in their arrangement or number. The chromosomes seem to be completely lost, as is usually the case in successive nuclei when there is a well-defined period of rest between the mitoses.

The cell-wall laid down after the first mitosis separates the

lobes of the spore-mother-cell into pairs which lie at an angle to one another if not actually perpendicular. The two spindles of the second mitosis are therefore not in the same plane, and a side view of one necessitates an oblique view of the other (see Fig. 14). These spindles present the peculiarity of bending into the form of a bow so that the poles may extend well into the lobes of the spore-mother-cell. This feature is shown in Fig. 13 as well as other characters. The spindle is entirely similar to that of the first mitosis, only smaller. It has the broad rounded poles ending in clear kinoplasm, and is composed of a dense mass of very delicate fibrillae.

Stages of prophase are very much less conspicuous than those of the first mitosis, but there is a well-defined condition when the zone of kinoplasm surrounding the daughter-nucleus becomes differentiated into a mass of fibrillae.

Following the metaphase (Fig. 13) the two sets of daughter-chromosomes come to lie in a region of kinoplasm clearly separated from the surrounding cytoplasm, at the poles of the spindle (see Fig. 14). In this area the daughter-nuclei are organized. A cell-plate appears in the equatorial region of the spindle, is then shortly replaced by a cell-wall, and the division of the spore-mother-cell is completed.

Because of the order in which these cell-walls are laid down the arrangement of the spores is not tripartite. However, the four portions diverge in such a symmetrical manner that sections frequently give the appearance shown in Fig. 15, but in this case it is plain that the first wall laid down runs from *a* to *b*. The nucleus in each spore immediately passes into a resting condition in which the chromosomes break up into granules that become distributed through the interior. The linin network does not appear until the nucleus becomes much larger and has taken its final position near the centre of the cell.

We will now consider the structure and behaviour of the chromosomes. They are eight in number in the mitoses of the spore-mother-cell and in the germinating spore, and sixteen

in all regions of the sporophyte that have been examined. The chromosomes are quite small in all mitoses except those of the spore-mother-cell and the first two divisions in the spore, which are consequently most favourable for study. When the nucleus of the spore-mother-cell emerges from synapsis (Fig. 3) there is presented a narrow much-coiled spirem-thread, bearing a line of granules that seem for the most part to be in a single row. The spirem shortens very much, and at the time of segmentation presents a very different structure. It is much thicker, and in side view appears as a band whose surface bears two rows of minute droplets (see Figs. 7 and 8). However, cross-sections of the thread or the chromosome-segments show that the form is not that of a band but rather of an angled bar, and that the chromatin-droplets are distributed with much irregularity. In Fig. 17 we have presented a segment of the spirem whose ends are bent towards the observer, and in Fig. 18 we have a selected group of chromosomes as seen in cross-section. The angled rod-like form with the seemingly irregular distribution of the granules is apparent from these sections and views. While it is quite possible that cross-sections may present groups of chromatin droplets in fours, as Guignard ('99) reports for *Naias* and Strasburger ('99) for a number of forms, the writer is convinced that no importance can be attached to such conditions in *Pellia*. There is no double longitudinal splitting of the mother-chromosome in *Pellia*, and the conclusions of Guignard ('99) and Strasburger ('99) cannot perhaps be applied as generally as we might desire. In this particular the present investigation conflicts with Farmer's ('94) account of *Pallavicinia*, where four chromatin droplets, by twice doubling, are said to give rise to sixteen chromosomes that then pass in groups of four to the poles of the quadripolar spindle. However, Farmer has shown that the mitoses in the spore-mother-cell of Hepaticae are usually successive, and he regards *Pallavicinia* as an exception to the general rule and an illustration of a process 'very much crowded up' ('94, p. 49).

The division of the chromosomes and their arrangement during metaphase of the two mitoses in the spore-mother-cell presents features well known to cytologists. Farmer described the first mitosis in several Hepaticae as heterotypic. The writer found no evidence of such conditions in *Pellia*. The chromosomes split longitudinally and, as they separate, present various figures in both mitoses according to the position at which the fibrillae are attached. Figs. 19 and 10 show the commonest manner of attachment and separation, the twisted daughter-chromosomes being pulled apart from their ends. Very much less frequently the fibrillae are attached in the middle region of the daughter-chromosome as in Fig. 20, and the elements might then present the form of a ring as they are drawn apart, which arrangement would be heterotypic. But the daughter-chromosomes are so much like short rods that there is little opportunity for complexity of arrangement at the time of separation, and the V figure prevails.

The chromosomes at the metaphase of mitosis are homogeneous in their make up, no granules being distinguishable in the structure, but a change comes over the elements during anaphase, and while the daughter-nuclei are being organized. The chromatin-droplets appear again in an irregular row (see Fig. 21), on the variously bent rods which shortly begin to fragment. In this manner the droplets of various sizes are set free in the interior of the daughter-nuclei (see Figs. 12 and 16).

We now pass to the consideration of the mitoses found in the germinating spore. This is a most interesting period in the life-history of *Pellia*, one very favourable for the study of nuclear phenomena, and most so during the first and second mitoses. The nuclei presented in later stages of germination are very much smaller, and peculiarities found at first are inconspicuous then. A paper by Farmer and Reeves ('94), 'On the occurrence of Centrospheres in *Pellia epiphylla*,' dealt with this period of ontogeny. They found centrospheres with radiations outside of and touching the

nuclear membrane. In some instances this structure appeared at a distance from the nucleus, but it was suggested that such examples resulted from sections across an unsymmetrical nucleus. Their material was killed in strong spirit.

These discoveries were well founded. There are aster-like structures in the cytoplasm, and they are frequently associated with a differentiated body that very strongly suggests a centrosphere. To the writer the problem of the significance of this manifestation is of some importance. A form which may have asters and centrospheres at one phase of ontogeny and entirely lack them at another, viz. in the spore-mother-cell, is deserving of careful study.

The nuclei that pass into the lobes of the spore-mother-cell are relatively small. They come to lie near the centre of the spore, and increase rapidly in size until they have attained three or four times their previous diameter, as may be seen by comparing Fig. 16 (Pl. X) with Figs. 22–25 (Pl. XI). At first there is little evidence of a linin network, and the chromatin lies in droplets or granules irregularly distributed in the nuclear cavity. Later, however, the amount of chromatin is very greatly increased, taking the form of large irregular bodies, which finally become arranged in a spirem. These characters of the nucleus are chiefly interesting to us at present as they may be correlated with the development and differentiation of the aster-like structures.

Whether these are true asters or not is a problem far too complicated for our present study, as it involves evidence from phylogeny. Certainly they have every outward appearance of typical asters, and it is only when we examine their origin, behaviour, and fate that we may feel justified in withdrawing such a designation. The writer does not believe that these structures remain permanently in the cell through successive mitoses, nor is he satisfied that they divide to determine the poles of nuclear figures. Such behaviour is generally expected of asters chiefly through investigations on animal forms, but botanists as well have reported such activities in detail, notably Swingle ('97) in *Sphacelaria* and

Mottier ('00) for *Dictyota*. Nevertheless, the writer holds the view that these structures in *Pellia* have a relation, although perhaps very distant, through phylogeny to typical asters, and they will be called such in this paper.

The first indication of an aster in the spore of *Pellia* is a differentiated area of kinoplasm near the nucleus, distinguished from the surrounding cytoplasm by its dense granular character, which stains deeply, its freedom from plastids, and the absence of alveolar structure. The writer believes that the asters arise independently of one another in two distinct regions of kinoplasm. They would not then be related as asters, although it is quite possible that their substance, the kinoplasm, may have come from the same source.

In justice, however, we should first note some facts that to many will seem strong evidence of behaviour quite the opposite to what has been suggested, and fully in accord with the activities expected of typical asters. It is not unusual to find the kinoplasm around a nucleus massed at one side in a very conspicuous manner. Such an example is shown in Fig. 22, a nucleus in synapsis just previous to division. However, the writer was never able to differentiate an aster in such a mass of kinoplasm, and in his studies has never found a nucleus with a clearly-defined solitary aster beside it. This is a very important point, and the search was persistent. The region of kinoplasm at the side of the nucleus may be large, but at this time it is homogeneously finely granular. The asters appear on opposite sides of the nucleus, sometimes applied to the nuclear membrane, sometimes at a distance from it.

Only one example was observed where the asters were separated by much less than 180° , and this specimen is shown in Fig. 23. In this case the distance between the asters is hardly more than 90° . To many the discovery of one such instance will entirely invalidate negative evidence, and they will hold that here is presented a stage in the separation of two daughter-asters that were derived from one. One

must frankly admit the justice of such criticism, and recognize the value of this cautious attitude. It is possible that the asters divide, and the evidence against it is purely negative, but the writer's disbelief is not founded on a hasty examination. There are also some peculiarities that should be noted in this connexion. The kinoplasm displays a remarkable degree of mobility in the various positions which it takes around the nucleus. We have noted the condition where it is massed on one side (Fig. 22), but usually the material is distributed more evenly. If a nucleus be elongated ever so little in any direction one may expect to find accumulations of kinoplasm at the poles. It is not necessary that such a nucleus be preparing for mitosis, although such accumulations of kinoplasm are associated with conditions of prophase. The kinoplasm usually lies close to the nuclear membrane, but it may sometimes be found in masses somewhat removed, and when such accumulations organize asters (see Fig. 25) they are at a distance from the nucleus. As a rule, however, the former conditions prevail, and kinoplasm before the differentiation of the aster appears as in Fig. 24, and after the development of these elements presents the structures shown in Figs. 26 and 27.

In the mobility of the kinoplasm we may have the explanation of the apparent development of two asters independently, and as far remote as possible from one another, sometimes touching the nucleus, and sometimes at a considerable distance from it (Fig. 25). Portions of the kinoplasm seem able to act in a great degree independently of other parts to accumulate material, and at the proper time organize asters.

Certain peculiarities of the aster should be noted before attempting to discuss its origin. The writer has preparations and is familiar with the asters of *Fucus*, *Sphacelaria*, and *Dictyota*, structures more clearly differentiated than the asters of *Pellia*. Of these three the aster of *Fucus* is perhaps the least sharply defined in its minute structure, although most conspicuous as a whole. The aster of *Pellia* resembles that of *Fucus* in that the radiations proceed from a rather vague

centrosphere-like region. But the radiations in *Pellia* are very irregular in distribution, and variable in number. They are also sometimes so coarse as to resemble strands of protoplasm rather than fibrillae. The centre of the aster is most clearly differentiated at periods previous to metaphase. At best it is a region of dense protoplasm, whose margin may stain somewhat deeply where the radiations have their origin. The interior of the structure is frequently granular, but generally homogeneously so, and there is no centrosome. These characters are shown in Figs. 28 and 29, the former being a view from above. Some granules are larger than others, but there is certainly no rule as to number or position.

But the degree of differentiation described above is frequently vague, and sometimes entirely lacking. The centre of the aster may be merely a region where a number of radiations converge, and without further form (Figs. 27 and 30).

As mitosis proceeds, the cytoplasmic radiations become less and less conspicuous, while the spindle-fibres extend and thicken. The former are generally absent at the metaphase, and the poles of the spindles (see Fig. 31) have a broad oval outline, and are usually entirely free from bodies that could suggest centrospheres or centrosomes. But it sometimes happens that the cytoplasmic radiations persist and form a bushy crown of fibrillae at the pole of the spindle, as is shown in Fig. 32. Such conditions remind one strongly of the aster in *Fucus*, but they are exceptional. Broad-poled spindles, quite lacking cytoplasmic radiations or other differentiations of kinoplasm, are the rule in the mitoses of the germinating spore.

With the anaphase the pole of the spindle becomes more pointed (see Fig. 33), the fibres apparently drawing away from the neighbouring cytoplasm so that there may be a clear area around the pole. This is the period when the pole is most pointed, but cytoplasmic radiations seem never to be present. All trace of the aster is lost.

The daughter-nuclei, when organized, lie surrounded by a zone of dense granular protoplasm, separating them from the

plastids and the usual cytoplasm with its alveolar structure. This zone of granular protoplasm is probably kinoplasm, but it fails to show further morphological differentiation. The writer searched in vain for the aster or centrosome that might be expected to accompany these resting nuclei.

As has been said, the asters are most conspicuous at the first mitosis in the spore. They are usually also prominent at the time of the second mitosis, but are not as large. Fig. 35 illustrates the appearance of these structures at the prophase of the second mitosis, when the amount of kinoplasm has been so much reduced that the asters would hardly be recognized were it not for the advantage of their positions. The condition here illustrated is rather exceptional for the second mitosis, as the appearance of the aster is generally more like Fig. 30, but it shows excellently the conditions that appear shortly, as nuclear division proceeds in the spore. As a rule, the aster disappears after the third mitosis. The nuclei decrease in size, becoming perhaps only one-third of their former diameter, and while this naturally makes the examination with respect to asters more difficult, nevertheless the conditions seem clear. The writer found no asters with the prophase of the later mitoses. The spindle seems to arise from an accumulation of kinoplasm that forms a cap over the end of the nucleus, a condition that will be described in greater detail in the account of mitosis in the stalk of the sporophyte.

Certain facts concerning the behaviour of the chromosomes in the mitoses of the spore are worth noting. The increase in the amount of the chromatin after the division of the spore-mother-cell is conspicuous, and results in the appearance of a linin network with deeply stained granules distributed over it. After a synapsis the linin and chromatin material takes the form of a broad spirem-thread. Synapsis is a phenomenon appearing with great regularity in the writer's preparations, not only before the first, but also before the second and later mitoses. It is difficult to think of it as an artifact when found in different phases of ontogeny side by side with other stages that seem well fixed.

At the time when the spirem-thread divides to form the chromosomes, the droplets of chromatin appear clearly in two rows along the side of the segments (see Figs. 26 and 27). The segments shorten up into rods, which clearly split longitudinally a little before their arrangement at the nuclear plate, and generally separate first at one end, where they are drawn apart, making a V-shaped figure. The dissolution of the nuclear membrane takes place after the spindle-fibres from the aster have grown down over it so as to form a sort of cap, as is shown in Fig. 30. When dissolved the fibres immediately enter the nuclear space (see Fig. 29), gathering the chromosomes together at the equator. There are no mantle-fibres, and generally very few radiations at the poles. The nucleolus disappears shortly before the metaphase, after developing a vacuolate structure and fragmenting. A new nucleolus is formed in the daughter-nucleus very shortly after mitosis.

The thin and delicate cell-walls dividing the spore are plainly derived from a cell-plate of the usual structure, formed between the daughter-nuclei.

There remain for our consideration the nuclear activities in another phase in the life-history—the vegetative cells in the stalk of the sporophyte. We shall thus have examined the nucleus in most of the important periods of growth and development, excepting, however, at the time when the gametes are formed. The activities of the cells in the seta remind one of the conditions in root-tips. We find a tissue with very little differentiation, almost all of whose cells divide frequently and irregularly.

The first indication of approaching mitosis is the elongation of a nucleus and an increase in the amount of chromatin, which takes the form of elongated bodies (see Fig. 38) that later unite into a much-coiled spirem. But this nucleus is not favourable for studies upon chromosomes, and its chief interest lies for us in the development of the spindle and the possibilities of centrosomes and asters.

One may frequently find structures that have the appearance

of asters, and such an example is shown in Fig. 36. The two bodies there figured were conspicuous in a cell that contained very little cytoplasm, so that the interior was chiefly given up to vacuoles. The arrangement of the radiating strands of protoplasm is very suggestive of an aster, and the association with an elongated nucleus about to enter prophase lends favour to such a possibility. But the writer does not believe that these structures have anything to do with asters. They are merely an exceptionally regular and delicate arrangement of strands of protoplasm passing from the periphery of the cell to the interior and converging to points near the ends of the elongated nucleus. Such arrangements are very frequent in the cells of the seta, and are generally so well defined that their character is unmistakable (see Fig. 37). They are the strands of protoplasm that swing the nucleus in the centre of the cell. Although usually broad and distributed irregularly as in Fig. 37, the strands may be so delicate and symmetrically disposed as to resemble an aster.

When the spindle is to be developed the protoplasm begins to collect at the poles of the elongated nucleus. There is little in the structure or morphology of these accumulations to indicate their kinoplasmic nature. Like the rest of the cytoplasm they are rather free from granules, and do not stain deeply. That these arrangements are kinoplasmic is shown by their further history. They form caps over the ends of the elongated nucleus, as is shown in Fig. 38. By changing from a granular to a fibrous condition the caps become the poles of the spindle. This history is then in essential agreement with the conclusions of Hof ('98) and Nemec ('99), who have so thoroughly studied spindle-formation in vegetative tissue, and especially root-tips, in a variety of forms.

After the dissolution of the nuclear membrane the spindle-fibres grow rapidly into the nuclear cavity, carrying the segments of the spirem-thread to the centre of the nuclear figure (Fig. 39). There the chromosomes, probably always sixteen in number, are found at the nuclear plate (Fig. 40).

The poles of the spindles, as shown in Figs. 39 and 40, are entirely free from radiations or bodies resembling centrosomes, and they may be broad so that the bundles of fibrillae stand out clearly from one another. The writer can find nothing in the events above described to justify a belief that an aster is really present in the vegetative cells of the seta. It cannot be found with the resting nucleus or at the poles of the spindle, and certain appearances during early prophase are readily explained by the peculiar arrangement of the protoplasm in the cell¹.

GENERAL CONSIDERATIONS.

We are hardly prepared as yet to draw general conclusions on the cytological conditions in the Hepaticae, but some peculiarities appear conspicuously, and certain possibilities may be noted.

It would seem, from the studies on *Pellia*, that the morphological manifestations of kinoplasm may be remarkably various in the same life-history. Indeed there seems to be no fixity of structure apart from the finely granular condition that precedes mitosis. It is true that we have in the delicate granules (microsomes) of this protoplasm the forerunners of fibrillae, and the latter in turn organize the asters, but it has been generally considered that the asters are to a great degree permanent in the cell.

We have examined three important phases in ontogeny of *Pellia*, (1) sporogenesis, (2) the germination of the spore, and (3) vegetative activities in the seta, but there has been no agreement in the form assumed by the kinoplasm during mitosis. In the first instance fibrillae, acting largely inde-

¹ Mr. Van Hook ('00) reports a centrosome with radiations in the archegoniophore of *Marchantia*. This structure was first visible in the prophase at the poles of the elongating nucleus, and was not observed after the radiations disappeared. The suggestion is presented that it is 'a temporary body.' In this respect it agrees with the centrospheres in the gametophyte of *Pellia* and in the kinoplasmic caps in the seta of the sporophyte. It seems probable that in Hepaticae centrospheres and kinoplasmic caps will be found to be related structures, the former but a more highly differentiated condition of the latter.

pendently of one another, organized the spindles in the manner characteristic of Pteridophytes and Spermatophytes. In the second an aster with a centrosphere is developed during the prophase, but becomes less prominent at the metaphase, and disappears before the daughter-nuclei are formed. In the third phase the kinoplasm forms two caps fitting closely over the ends of the elongated nucleus, and these are changed into the poles of the spindle. Fibrillae, centrospheres, and the kinoplasmic caps, however they may be arranged, are all secondary developments from the primal granular protoplasm which is the only form of kinoplasm in any sense permanent in the cell.

Arising from accumulations of granular kinoplasm during the prophase, the substance of the fibrillae, centrospheres, or caps returns to a similar condition at the completion of mitosis, or is lost in the general cytoplasm of the cell. We do not yet know the structure of the nuclear figures when sexual cells are organized, or during the later periods of the gametophyte's history, but it is not unreasonable to expect a blepharoplast at the time of spermatogenesis. However, as nuclear division proceeds in the spore the centrosphere becomes less conspicuous until it cannot be distinguished, and the kinoplasm takes the form of a cap. It seems probable, therefore, that mitosis throughout most of the period of the gametophyte takes place, with spindles organized from caps of granular kinoplasm, as is the case in the seta.

The bearing of these data on the probable evolution of mitotic phenomena in higher plants from conditions in the Thallophytes is most interesting. We cannot trace the centrosphere or the aster phylogenetically back to the Thallophytes, for neither remains throughout the ontogeny of *Pellia*. We do not know the facts for the Algae most closely related to the Hepaticae (higher types of the Chlorophyceae, such as *Coleochaete*), but even if it be found that these forms have centrospheres and asters we shall still have a puzzling problem before us. For a connexion between such structures and the conditions in the Hepaticae may only be followed through

the undifferentiated granular kinoplasm, whose accumulation is the first indication of the approaching development of a spindle in this group.

In this connexion it should be noted that the relation of the aster to ontogeny is variously reported in the Thallophytes. Swingle ('97) was able to follow the aster from cell to cell in *Sphacelaria* with the probability of its being a permanent organ. Similarly, Mottier ('00) has recently traced the division of the aster in the tetrasporangium and vegetative cells of *Dictyota*. Strasburger ('97) has described the division of the aster in the germinating oospore of *Fucus*, and Harper ('97) followed the division of the centrosphere and aster during mitosis in the ascus. However, Farmer and Williams ('96 and '98) believe that the aster disappears with the anaphase of each mitosis in the oogonium of *Fucus*, and that a new structure is organized for the next division. My own examination of *Corallina* ('98) led me to similar views for the centrosphere-like aggregations present during mitosis in the tetrasporangium. In our ignorance of other Thallophytes, we are left in the perplexing situation now presented in zoology, where an increasing number of investigations have thrown considerable doubt on the older view of the permanence of the centrosome and aster in the cell.

It seems quite possible that we shall be forced to the conclusion that centrosomes, centrospheres, and asters are but secondary developments of the granular kinoplasm from which they sometimes, and perhaps frequently, arise. In such case they cannot be considered as strictly homologous structures, even in closely related plants, for phylogenetic connexions may be traced only through the granular kinoplasm.

The conditions in *Pellia* strongly support the celebrated archoplasm-hypothesis of Boveri, and accord well with Strasburger's conception of the structure and behaviour of kinoplasm. In *Pellia* the kinoplasm (archoplasm) consists of minute granules (microsomes), and this structure presents the possibilities of such fibrillar differentiation as may appear during mitosis in various phases of ontogeny. But the fibrillar

condition is nowhere permanent, but constantly returns to the granular state. The fibres then break down or are gathered up into some region of the cytoplasm which may have form, as at the cell-plate, but more frequently is a vague area near the nucleus.

It is interesting to consider the bearing of these studies on theories of the physiology of mitotic phenomena. The facts seem to support the view of fibrillar growth and contractility. The spindle-fibres certainly enter the nuclear cavity, and the nuclear plate takes its form in consequence. Groups of fibres are plainly attached to the daughter-chromosomes, and probably contract, but it is not easy to demonstrate this point. However, the formation of the cell-plate gives strong evidence of contraction, as the spindle shortens and the fibres, drawing away from the poles, contribute their substance to the protoplasmic membrane that foreshadows the cell-wall.

While growth and contractility are characteristic activities of the fibrillae, one must be impressed with the lack of evidence that these functions in *Pellia* are in any way controlled by morphological centres. Indeed it is even hard to believe in dynamic centres, so variable is the form of the kinoplasm. Yet dynamic centres may of course be conceived whose areas are comparatively ill-defined, and such may be presented by the centrospheres and by the vaguer kinoplasmic caps.

But these are mere conjectures, and it is best to face the fact that we have little else upon which to construct our idea of the physiology of the achromatic portion of the nuclear figure than the conception of kinoplasm with its power of forming contractile fibrillae.

SUMMARY.

At the time of sporogenesis the nucleus comes to occupy the geometrical centre of the four-lobed spore-mother-cell, and there passes through synapsis previous to the first mitosis.

Emerging from synapsis the nucleus presents a delicate

spirem-thread, which soon segments into the chromosomes. The thread bears granules irregularly disposed in two rows.

The first indication of spindle-development appears in a zone of granular kinoplasm closely investing the nucleus. Fibrillae are developed in this zone, appearing most numerous in the four regions where the lobes of the spore-mother-cell diverge from the centre. As the nuclear membrane fades the fibrillae enter the cavity, usually from more than two points.

At this period of mitosis an appearance is frequently presented similar to Farmer's ('94) account of a four-poled spindle, there being four conspicuous sets of fibrillae running into each lobe of the spore-mother-cell. This is however a prophase of mitosis, and all trace of these four regions of fibrillae is lost with the metaphase. There is no four-poled spindle.

The mitoses in the spore-mother-cell are two in number, and successive, with a short period of rest between the first and the second. The spindles are remarkable for the great breadth of their poles and the number and delicacy of the fibrillae that traverse the axes. The poles of the spindles are never occupied by bodies comparable to centrospheres or centrosomes. On the contrary the development of the spindle follows closely the plan generally presented by Spermatophytes and Pteridophytes, the fibrillae acting individually or in groups or bundles, to a great degree independently of one another and uncontrolled by fixed centres.

The rod-shaped chromosomes split longitudinally in each mitosis. There is no premature division of the mother-chromosome by double longitudinal splitting to give four daughter-chromosomes, such as has been reported by Guignard ('99) and Strasburger ('99) for several Spermatophytes. On the contrary the daughter-nuclei, following the first mitosis, pass into a resting condition, and the chromosomes, breaking up into granules, seem to lose their form and cannot be distinguished.

The cell-walls separating the spores are laid down from cell-plates formed with each nuclear figure in the manner

usual in Pteridophytes and Spermatophytes, and bear no analogy to the conditions reported by myself for *Anthoceros* (Davis '99) which have recently been explained by Van Hook ('00).

Following the mitoses in the spore-mother-cell, each nucleus takes a position in the centre of the spore, assuming a resting condition. It increases in size and soon prepares for division, passing through synapsis.

The kinoplasmic activities associated with the first two or three mitoses in the spore are very conspicuous. Two centrospheres with radiations resembling asters are organized during the prophase, and these develop the poles of the spindle. It is the writer's opinion that these structures are developed *de novo* from accumulations of granular kinoplasm. He was not able to satisfy himself that they remained permanently through successive mitoses or ever divided. They appear on opposite sides of the nucleus, are most prominent during early prophase, and frequently disappear before the metaphase, leaving a blunt-pointed spindle ending in a region of granular protoplasm. There is no trace of the centrosphere and aster at the anaphase or beside the resting nucleus. The substance of the aster not used in forming the spindle returns to the granular condition. The aster becomes small after the second mitosis in the spore, and cannot be clearly distinguished in later divisions.

The aster in the spore of *Pellia* resembles that of *Fucus* in that the radiations proceed from a vague centrosphere-like region, but they are not as numerous nor as regular in their distribution, and are sometimes so coarse as to resemble strands of protoplasm rather than fibrillae. The interior of the centrosphere is generally homogeneous, containing no centrosome.

The relation of the centrosphere to the spindle is clear. The membrane breaks down at the ends of the nucleus, the spindle-fibres growing into the nuclear space from the centrospheres, gathering the chromosomes at the equatorial plate.

Mitosis in the seta of the sporophyte recalls the nuclear

activities reported by Hof ('98) and Nemec ('99) in root-tips. The first sign of approaching division is the elongation of the nucleus and development of the spirem-thread. Kinoplasmic accumulations then appear, forming two caps over the ends of the nucleus, and these organize two sets of fibrillae which grow into the nuclear cavity carrying the chromosomes to the nuclear plate. Certain arrangements of the cytoplasm sometimes suggest asters, for the nucleus is swung in the centre of the cell by strands of protoplasm, but such appearances are deceptive. The poles of the spindles are entirely free from radiations or bodies comparable to centrosomes.

There are eight chromosomes in the gametophyte and sixteen in the sporophyte.

The most interesting results of these studies are perhaps the evidences of the various morphological manifestations of kinoplasm possible in the same life-history. The granular kinoplasm organizes (1) independently-acting fibrillae in the spore-mother-cell, after the manner in Pteridophytes and Spermatophytes, (2) a centrosphere and aster in the germinating spore which (3) give place to caps in older periods of the gametophyte, and (4) caps are developed in the tissue of the seta similar to those in root-tips. It is probable, of course, that there is likewise a blepharoplast at the time of spermatogenesis.

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June, 1900.

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EXPLANATION OF FIGURES IN PLATES X AND XI.

Illustrating Dr. Davis's *Nuclear Studies on Pellia*.

The preparations were studied with a Zeiss oil immersion 2 mm. (aper. 1.30) with compensating oculars. The figures were sketched under an Abbé camera with the following magnification: Figs. 1-3, 9, and 15, 500 diameters; Figs. 4-8, 10-14, 16, and 22-40, 1000 diameters; Figs. 17-21, 1500 diameters. Material

shown in Figs. 3-22 and 33 was fixed by the weak formula of Flemming; that in Figs. 1, 2, 23-32, and 34-40 by 1 % chrom-acetic acid. Figs. 1-6, 8, 10, 14-16, 18-20, 22-25, 29, 31-33, 35, 36, and 38-40 from sections $7.5\ \mu$ thick, and stained on the slide with safranin and gentian violet; Figs. 7, 9, 11-13, 17, 21, 26-28, 30, 34, and 37 stained with iron-alum haematoxylin after the method of Heidenhain.

PLATE X.

Figures 1-21, from the spore-mother-cell.

Fig. 1. Portion of spore-mother-cell, nucleus passing to the centre of convergence of the four lobes.

Fig. 2. Nucleus taking position at the junction of the four lobes.

Fig. 3. Synapsis occurring shortly before the first mitosis. Granular protoplasm (kinoplasm) lying next the nuclear membrane.

Fig. 4. Spirem-thread, nuclear membrane faint, granular kinoplasm around the exterior.

Fig. 5. Prophase: kinoplasm developing fibrillae which are converging towards the four lobes of the cell, making the nucleus four-angled and giving an appearance suggestive of a four-poled spindle.

Fig. 6. Prophase: fibrillae growing into the nuclear space; chromosomes organizing.

Fig. 7. Late prophase: nuclear membrane almost entirely dissolved, and nuclear space largely filled with developing fibrillae; chromosomes with two rows of granules.

Fig. 8. Surface view of nucleus in prophase, showing fibrillae extending into one of the lobes of the cell; chromosomes below with two rows of granules.

Fig. 9. Spindle at first mitosis showing broad poles; one of them concave so that part of the spindle-fibres lies in one lobe and part in another.

Fig. 10. First mitosis: daughter-chromosomes about to separate at the nuclear plate; broad spindles with rounded poles, the fibrillae ending in granular kinoplasm.

Fig. 11. Formation of cell-plate after first mitosis; spindle-fibres appearing to contract from the region of the daughter-nuclei; no aster or radiations beside the daughter-nuclei.

Fig. 12. Daughter-nuclei after first mitosis; chromosomes no longer present as organized bodies, but in their place a linen network with numerous granules; several nucleoli; nuclei lying in a bed of granular protoplasm free from plastids, similar to that which formerly surrounded the mother-nucleus as in Figs. 3 and 4.

Fig. 13. Side view of spindle during second mitosis; slightly bent so that the poles enter the two lobes of the spore-mother-cell; poles broad and ending in granular kinoplasm without further differentiation.

Fig. 14. Anaphase of second mitosis, one spindle seen from the side, the other viewed obliquely from above; chromosomes passing into an area of granular kinoplasm.

Fig. 15. Nuclei in spores after the second mitosis; clear protoplasm at base of the spore derived from material associated with the two spindles.

Fig. 16. Nucleus in spore shortly after the second mitosis; chromosomes fragmented into granules; early appearance of a nucleolus.

Fig. 17. Chromosomes at beginning of prophase; granules arranged irregularly in two or more rows.

Fig. 18. Chromosomes at time of prophase, in section showing irregular distribution of granules.

Fig. 19. Daughter-chromosomes pulled apart from the ends.

Fig. 20. Daughter-chromosomes pulled apart from the middle region.

Fig. 21. Chromosomes breaking up in the daughter-nucleus after the first mitosis.

PLATE XI.

Figures 22–35, from germinating spores.

Fig. 22. Nucleus in spore; synapsis previous to first mitosis; aggregations of kinoplasm at one side of nucleus suggests an aster, but is without characteristic differentiation.

Fig. 23. Two asters closely applied to the nucleus, the only example observed where such structures were much nearer than 180° .

Fig. 24. Kinoplasmic differentiation at poles of an elongated nucleus entering prophase, as yet scarcely having the characters of asters.

Fig. 25. Two asters at a distance from the nucleus, the latter not yet in prophase.

Fig. 26. Asters developing from aggregations of kinoplasm; spirem-thread segmented.

Fig. 27. Fibrillae from aster growing around the end of the nucleus.

Fig. 28. Aster viewed from above, showing insertion of fibrillae in an undifferentiated centrosphere-like body.

Fig. 29. Spindle-fibres growing into the nuclear space, centrosphere-like mass of kinoplasm at the poles.

Fig. 30. Spindle-fibres arising from a region of kinoplasm without evidence of an aster-like structure.

Fig. 31. Spindle at metaphase with no trace of aster at the poles, fibrillae running into granular kinoplasm.

Fig. 32. Pole of spindle at metaphase with bushy aggregation of fibrillae.

Fig. 33. Pole of spindle at anaphase, fibres coming to a point in a region of finely granular kinoplasm.

Fig. 34. Organization of daughter-nuclei, no aster present.

Fig. 35. Synapsis and prophase of a later mitosis in the germinating spore, showing the small asters and delicate radiations in a less amount of kinoplasm.

Figures 36–40, from seta of a young sporophyte.

Fig. 36. Arrangement of delicate strands of cytoplasm resembling an aster.

Fig. 37. Kinoplasm assembling at the ends of the nucleus; stellate arrangement of the cytoplasm; strands much broader than in Fig. 36.

Fig. 38. Kinoplasm forming a cap at the poles of the elongated nucleus that is entering prophase.

Fig. 39. Spindle developed from the caps of kinoplasm; chromosomes being carried to the nuclear plate.

Fig. 40. Spindle at metaphase, showing poles formed by convergence of bundles of fibres and without a centre.

Fig. 1.

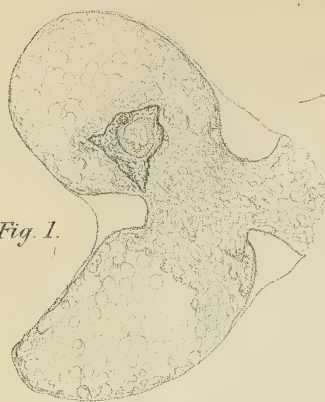


Fig. 2.



Fig. 3.

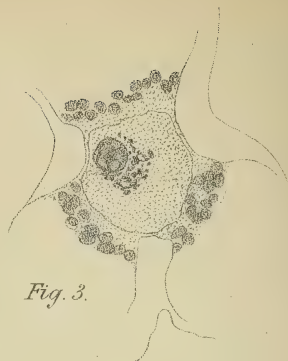


Fig. 7.



Fig. 6.

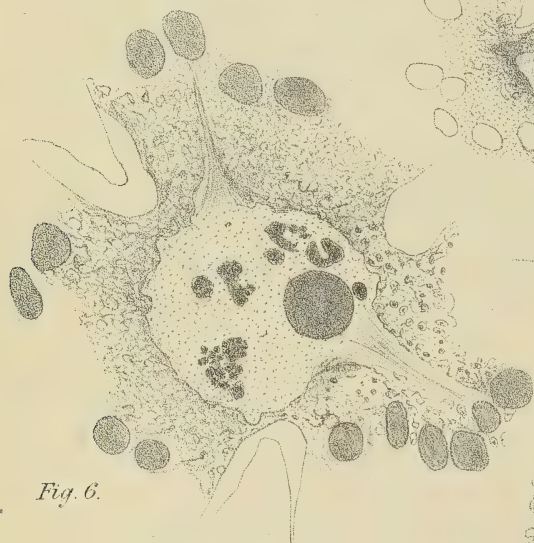


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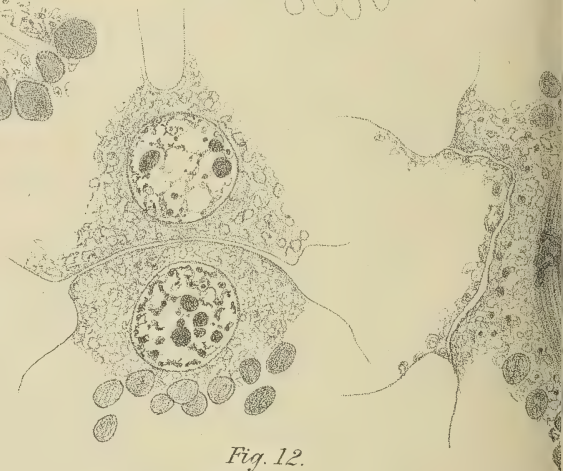


Fig. 11.

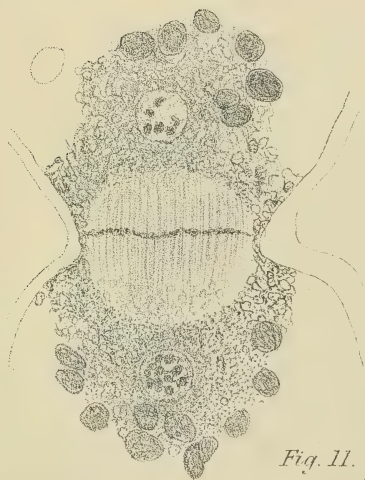


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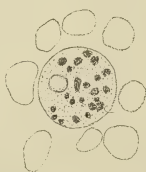
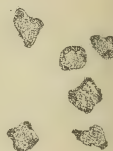


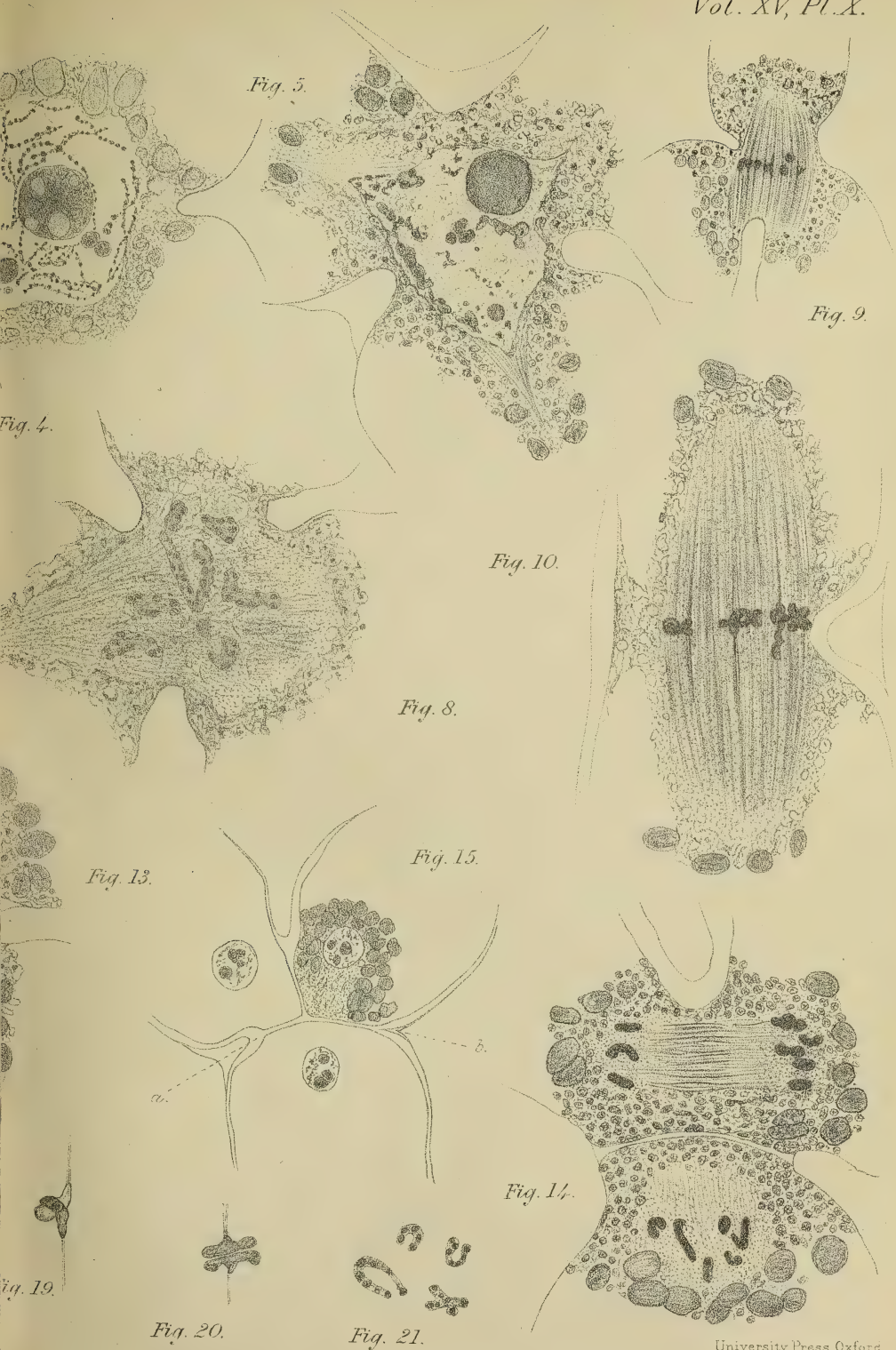
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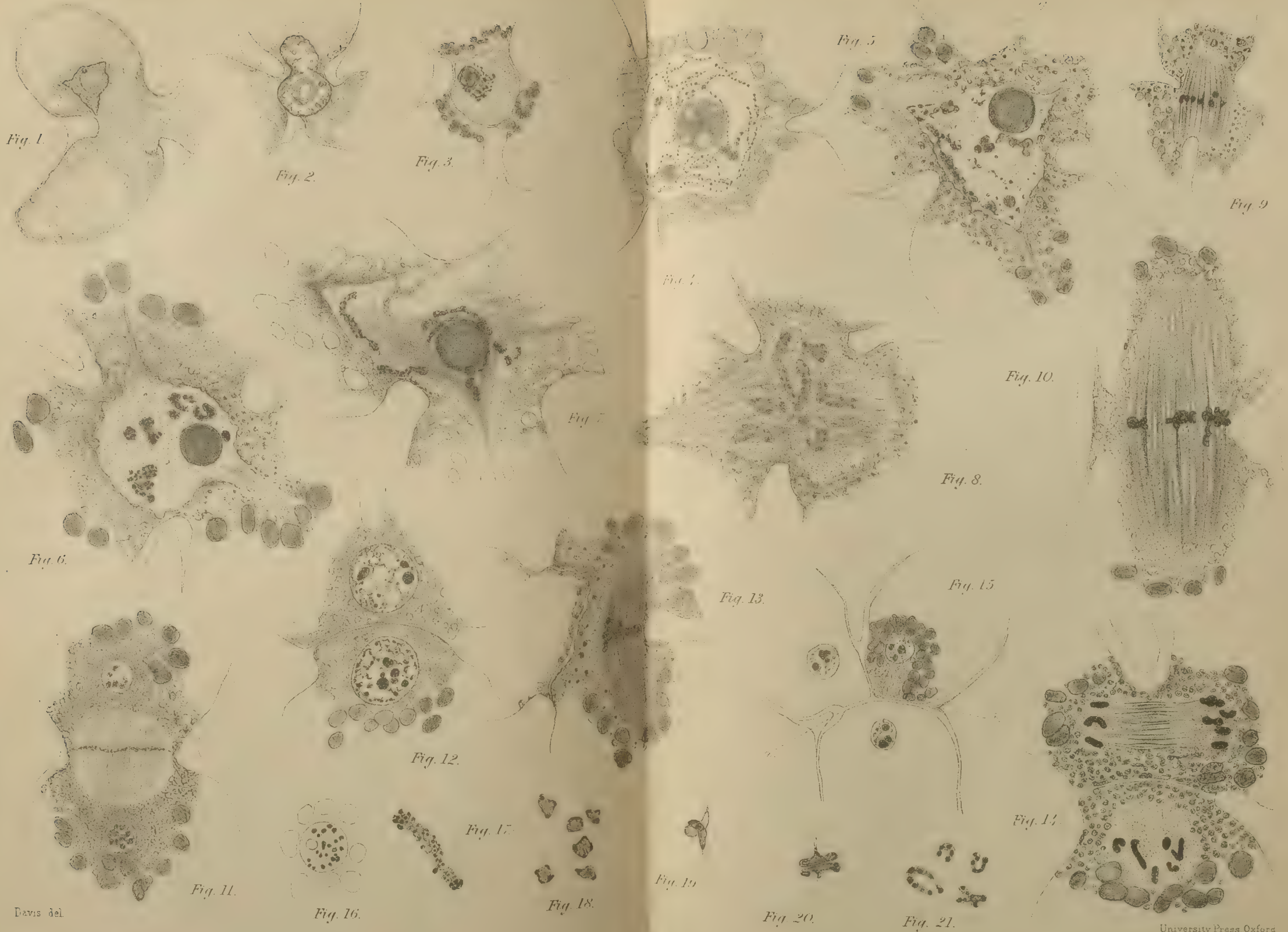


Fig. 18.



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Fig. 22.

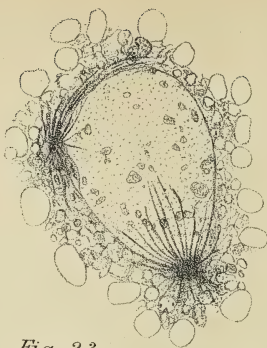


Fig. 23.



Fig. 24.

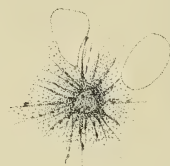


Fig. 28.



Fig. 29.

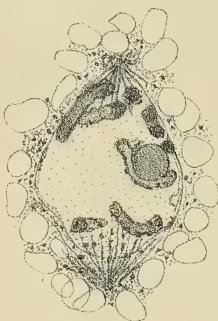


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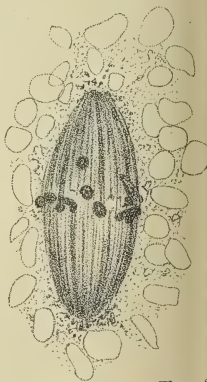


Fig. 31.



Fig. 35.



Fig. 36.



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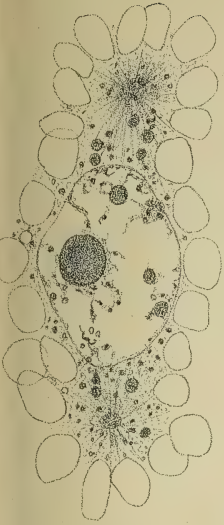


Fig. 25.



Fig. 26.

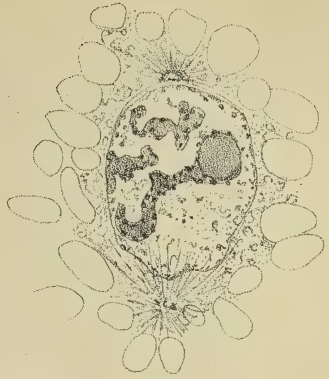


Fig. 27.



Fig. 32.

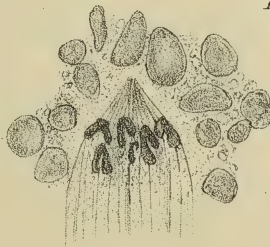


Fig. 33.

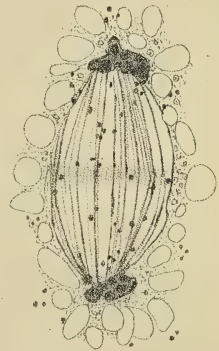


Fig. 34.



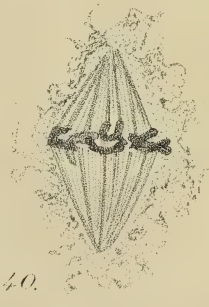
Fig. 38.



Fig. 39.



Fig. 40.



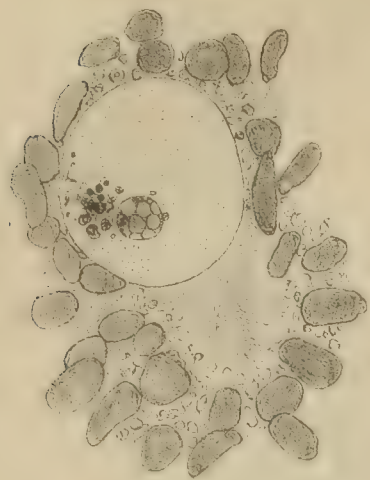


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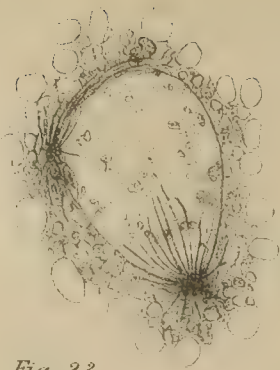


Fig. 23.

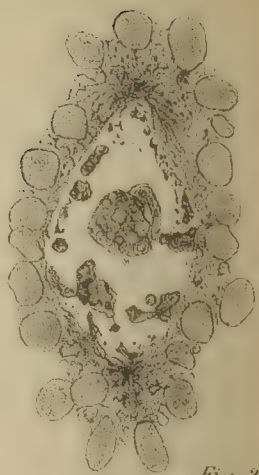


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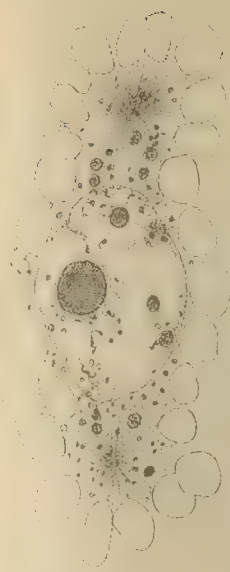


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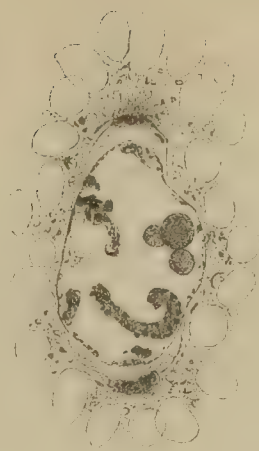


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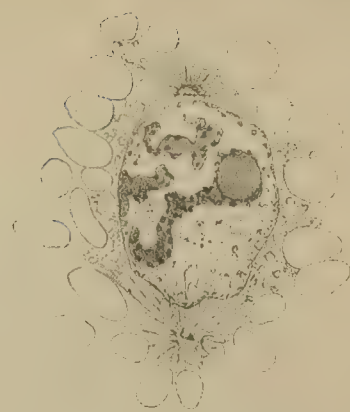


Fig. 27.



Fig. 28.

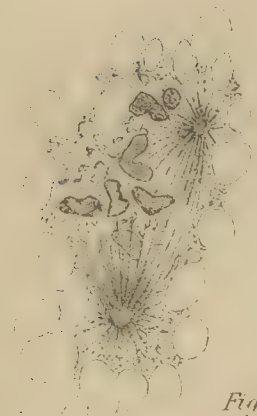


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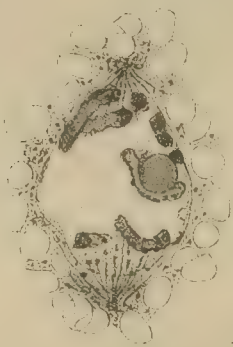


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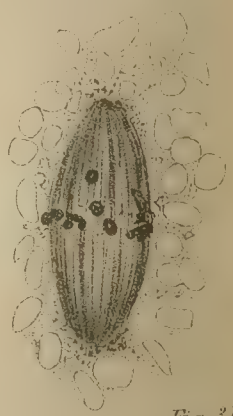


Fig. 31.



Fig. 32.

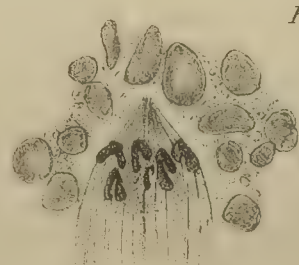


Fig. 33.

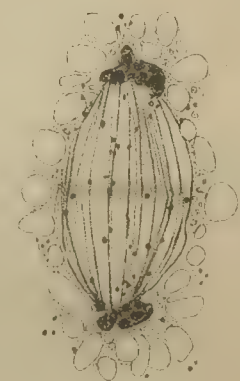


Fig. 34.



Fig. 35.

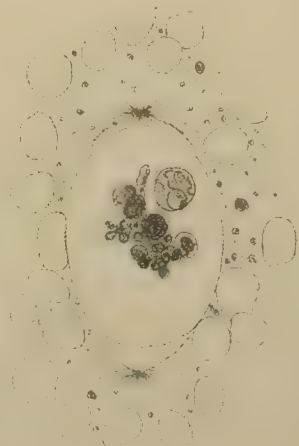


Fig. 36.



Fig. 37.



Fig. 38.



Fig. 39.

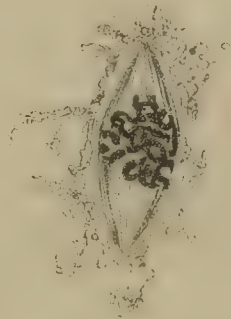


Fig. 40.



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NOTES.

ON LEPTOMIN.—About forty years ago, Schönbein¹ discovered that various parts of plants contain substances which give certain reactions with tincture of guaiacum : (1) substances (or a substance) which cause the guaiacum to turn blue immediately on contact ; (2) substances (or a substance) which only cause the guaiacum to turn blue in the presence of hydrogen peroxide. The substances of the first class are those which are now generally known as *oxydases* : but it is not with these, but with those of the second class that I am now concerned.

Schönbein enumerates a number of vegetable substances which give the blue reaction with tincture of guaiacum to which hydrogen peroxide has been added : such are gluten (from wheat-flour), diastase, emulsin, myrosin, yeast, watery extracts of various seeds (barley, oats, millet, poppy, cress) and of various roots (Dandelion, Lettuce), as also of potatoes.

Although attention was drawn to this subject by Jamieson² in 1878, it was not until the recent publication of Raciborski's observations that it came prominently into notice. Raciborski³ has proved the presence of a substance giving this reaction, not only in vegetable liquids, such as coco-nut milk and latex, but also in various tissues, such as the endodermal passage-cells in the aerial roots of Orchids, the lenticels, the spongy parenchyma of aquatic Phanerogams, and more especially the sieve-tubes of a number of plants. It is, in fact, to emphasize the occurrence of this substance in the phloem that he has given to it the name *leptomin* ; a designation which seems to be misleading, inasmuch as it suggests that the substance in question is in some special way associated with the sieve-tissue, whereas his own researches prove that it is widely distributed in the tissues and fluids of plants.

Raciborski's suggestion as to the probable physiological significance of 'leptomin' is perhaps even less justifiable than his nomenclature.

¹ Katalytische Wirksamkeit organischer Materien und deren Verbreitung in der Pflanzen- und Thierwelt : Journ. f. prakt. Chemie, 89, 1863.

² Nature, vol. xviii, 1878.

³ Ber. d. deutsch. bot. Gesellschaft, xvi, 1898 : Flora, 85, 1898.

Since the time of Schönbein it has been known that blood, or more particularly haemoglobin, has the property of causing guaiacum to turn blue in the presence of hydrogen peroxide. On the strength of their similarity in this respect, Raciborski goes so far as to state that 'leptomin performs, in vascular plants, a function analogous to that of haemoglobin in the higher, and haemocyanin in the lower, animals, in that it is a vehicle charged with oxygen maintaining internal respiration, that is, a supply of oxygen from the phloem and the laticiferous system to the adjacent tissues.'

Now it is well known that haemoglobin possesses in a high degree the property of combining with free oxygen and of readily parting with it: but it is not clear that this property is expressed by, or indeed stands in any relation with, its power of decomposing hydrogen peroxide. For many tissues of the animal body, as also freshly prepared blood-fibrin, have been found to decompose hydrogen peroxide, without there being any suggestion that they are, like haemoglobin, of physiological importance as oxygen-carriers. This is precisely the position of the matter as regards leptomin. Because it decomposes hydrogen peroxide, it does not follow that it is an oxygen-carrier like haemoglobin; there is no evidence whatever to show that it shares in any degree the property of combining with oxygen which is so characteristic of haemoglobin. Nor is there any ground for distinguishing physiologically, as Raciborski apparently does, between the 'leptomin' of the phloem and the laticiferous tissue, and the 'leptomin' of coco-nut milk or of potato pulp.

The analogy which Raciborski seeks to establish between 'leptomin' and haemoglobin, attractive as it is, would seem to be premature, to say the least. The fact that there exist, in the body of both animals and plants, substances which can decompose hydrogen peroxide, still awaits, I believe, its physiological interpretation; and its significance is rendered all the more problematical by the great improbability of the occurrence of hydrogen peroxide in the living organism. It is, however, possible to conceive that unstable, highly oxidized, organic substances may be formed and distributed within the organism, and that their loosely combined oxygen can only be made available in the tissues on decomposition by the substances which we now only know by their action on hydrogen peroxide; it is not inconceivable, for instance, that the decomposition of oxy-haemoglobin may be effected in this way.

To facilitate repetition of the experiments, I give a few details as to the application of the guaiacum test. The tincture is prepared by dissolving the resin in absolute alcohol, to a deep brown solution: fresh tincture should be made from time to time, as it is liable to lose with age its property of turning blue when oxidized, especially if it be exposed to light. When the test is about to be applied, a small quantity of hydrogen peroxide, not enough to cause a permanent precipitate, should be added to some of the guaiacum tincture. A few drops of the mixture are then poured on to some of the liquid to be tested, either in a test-tube or on a piece of white porcelain. Boiling destroys the activity of all vegetable liquids. With regard to testing for 'leptomin' in the tissues, the tissue must first of all be freed from oxydases, as Raciborski points out, either by heating to 60° C., or by keeping it for a time in absolute alcohol: but the stay in alcohol must not be too long, otherwise the property of decomposing hydrogen peroxide will also be destroyed. The tissue is then to be treated with the mixture of guaiacum tincture and hydrogen peroxide, when a blue colour will appear in the tissues containing 'leptomin.'

I may add, in conclusion, a few further observations of my own as to the distribution of 'leptomin,' or bodies of that class, in plants. I find that pine-apple juice gives a strong reaction, whilst the juice of oranges, lemons, and apples gives no reaction: the pulp of the apple, and still more its rind, gives a slight reaction. Non-drying oils, such as olive, almond, colza, and rape, give the reaction, but not the drying oils, such as linseed, walnut, and poppy. The behaviour of different kinds of wheat-meal is striking: bran gives a good reaction when a little is sprinkled on a few drops of the mixture of guaiacum and hydrogen peroxide; 'toppings' and 'middlings' give a strong reaction, whereas the pure flour gives little or no reaction.

S. H. VINES.

OXFORD.

ON THE ANATOMY OF THE STEM OF DALBERGIA PANICULATA, ROXB.—For some time it has been known that the stem of *Dalbergia paniculata*, Roxb. exhibits certain anomalies as to its structure. Brandis¹ writes thus concerning this plant:—'A

¹ Brandis, Sir Dietrich, *The Forest Flora of North-west and Central India*: London, 1874, p. 151. See also Gamble, J. S., *A Manual of Indian Timbers*: Calcutta, 1881.

moderate-sized or large tree (60 ft. high in the Satpuras). Trunk erect, irregularly scooped out, fluted and compressed, attaining 5-6 ft. girth. Bark smooth, greenish white, no heart-wood. Structure most remarkable, entirely different from that of other *Dalbergias*. Broad concentric masses of wood alternate with narrow soft layers of a fibrous substance, so that planks cut off old trees often fall to pieces.

'South and Central India. Gonda forests in Oudh. Silwalik tract west to the Jumna, ascending to 2,500 ft.'

As far as has been ascertained, no detailed observations have been carried out regarding the anomaly; and this being so, the writer was asked by Sir Dietrich Brandis to investigate the anatomy of the stem. The only material available for this was a dried portion of the trunk from a fair-sized tree. This piece measured 3 cm. in the radial direction, including the cortex, and exhibited two separate arcs of the anomalous structure, with a part of a third. The thicker of these two phloem-zones measured 1-2 mm. across, and the thinner .5-1 mm.

Inasmuch as the *raison d'être* of this note lies in the anomalous structure of the stem, it will be well to describe the anatomy of the narrow zones first. These rings, as has already been mentioned above, alternate with broad yellowish masses of xylem. By the examination of a transverse section, it may at once be seen that the narrow abnormal zones are of the nature of phloem, which is accompanied by a certain amount of cambium. This cambium is situated on the side nearer the centre of the stem, and abuts directly on the xylem-elements. Judging from the crushed appearance of the softer tissue nearer the periphery of the bast-rings, growth goes on to a fairly great extent, new elements being added by the activity of the cambium to the wood on the one side and the phloem on the other; thus a well-marked radial arrangement between these three tissues is seen.

Immediately succeeding the cambium, sieve-tubes and phloem-parenchyma occur, and in the more peripheral regions a large amount of sclerenchyma is produced. The sieve-tubes are extremely well shown, having sieve-plates which, in the material examined, are much obliterated by callus. Obliteration was only observed in those elements nearer the cambium, the sieve-tubes in the more external regions of the zones not showing callus: hence they are somewhat difficult to make out. The diameter of the sieve-plates is, on the average, about .03 mm., it is thus seen that they are of more than

average size. In longitudinal sections it may be observed that sieve-areas are quite numerous on the vertical walls of the tubes; these areas are especially well seen in those elements just external to the cambium, on account of the development of callus.

Sclerenchyma is formed at a short distance from the cambium. The elements of this hard bast are aggregated into masses which are somewhat regularly arranged in concentric rings, each being interrupted by the medullary rays. Alternating with these broken circles of sclerenchyma are soft bast elements which gradually become less in amount as the outer zone of wood is reached, so that in the peripheral parts of the anomalous rings of bast the sclerenchymatous bands may be separated one from the other apparently only by a thickness of one or two parenchyma-cells.

The individual elements of the hard bast do not call for much attention, inasmuch as they present the characters usually associated with such cells. Suffice it to say that they have very hard lignified walls with simple pits and, generally speaking, a very small lumen. For the sake of completeness it will be well to give a short description of the xylem. The wood is made up of the usual elements; that is to say, vessels, fibres, and parenchyma. The vessels vary much in diameter; in transverse section they are generally oval in shape. Very frequently the transverse walls of the original tracheides persist to a greater or less extent, in which case the remnant of the septum is much pitted. Xylem-parenchyma surrounds the vessels. The parenchymatous elements and the fibres are arranged in a somewhat regular manner in alternating zones. The cells constituting both these tissues have fairly thick lignified walls, those of the fibres being thicker than those of the parenchyma, and having simple pits.

The medullary rays are narrow, generally from one to four cells in breadth. They are numerous, and the elements constituting them are simply pitted. From the nature of the material—a dried piece of old stem—it was impossible, unfortunately, to determine the exact mode of origin of the anomaly described above. It appears very probable however that it arises in a manner analogous to that of the phloem-islands in *Strychnos*, for example; that is to say, by the formation of successive cambium-rings. It is hoped to further investigate the origin of the phloem-zones when fresh material is obtained.

It is not proposed to enter into theoretical considerations con-

cerning the facts embodied in this note. It is certainly extremely interesting and equally surprising to find a tree, attaining the height of 60 feet, exhibiting a structure such as is described above. A certain amount of light may be thrown on the subject by the fact that some species of the genus are lianes, and, what is more interesting, that one at least, *D. variabilis*, is a climber when growing in the forest, but a shrub when situated in the open.

Schenck¹ makes a few remarks concerning the anomaly of this plant. He states that within the genus *Dalbergia*, anomalous secondary thickening is only known to him in one species, namely *D. paniculata*. He then goes on to say that 'according to a statement of Brandis (Forest Flora), verified for me by the author, the stem of this species consists of broad concentric masses of wood, which alternate with narrow zones of phloem. This occurrence is the more remarkable, because *D. paniculata* is in no way a liane, but is a tree 60 ft. high. The question as to the origin of the anomalous structure is an open one. The possibility is not excluded, that this species may have been derived from a liane and may have inherited from the latter and retained as a character the special form of secondary thickening. Perhaps one will obtain data for the discussion of this question when the stem-structure and the precise affinities of all the species of *Dalbergia* are known. Gamble mentions besides the anomalous *D. paniculata*, Roxb. a number of East Indian species of *Dalbergia*, mostly trees (*D. Sissoo*, *latifolia*, *cultrata*, *lanceolaria*, *nigrescens*), and two lianes (*D. stipulacea*, *foliacea*), all of which exhibit normal wood.'

THOMAS G. HILL.

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THE OVARY OF PARNASSIA PALUSTRIS, LINN.—The following sentence in Payer's *Traité d'Organogénie comparée de la Fleur* (Paris, 1857), p. 183, first attracted my closer attention to *Parnassia palustris*:—

'Contrairement à ce qui se passe dans la plupart des autres plantes, cette fleur du *Parnassia palustris* commence par être irrégulière dans son développement et finit par être complètement régulière à son état parfait.' After satisfying myself that this was so, I examined a large

¹ Schenck, *Anatomie der Lianen*: Botanische Mittheilungen aus den Tropen, Heft 5, Theil 2, pp. 169–170.

number of flowers, and recorded the variation found to exist¹. In doing this I noticed that the traces of zygomorphy do not always completely disappear from the ovary. The present note is concerned with this entirely.

Nearly mature fruits were gathered on the Scarborough cliffs in 1895, and put into alcohol to harden. Some were examined after being in alcohol for six months; others—owing to other more pressing work—have remained in it for more than five years.

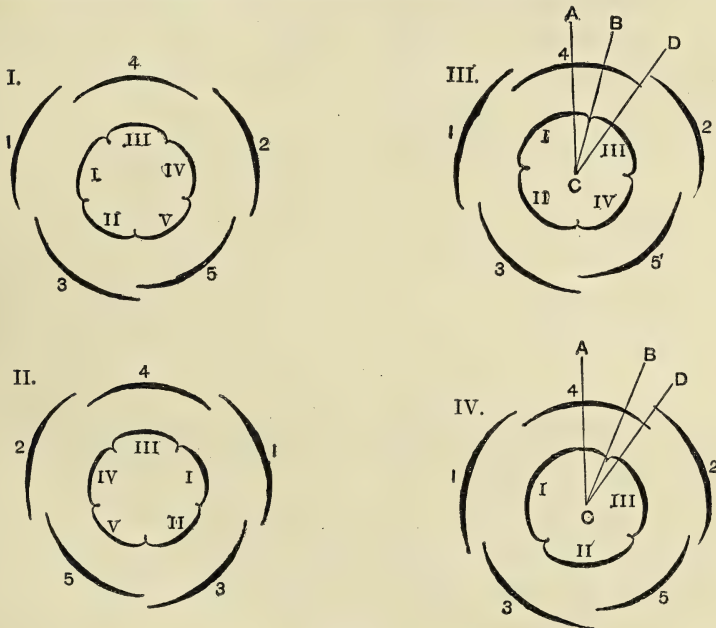


FIG. 1.

- DIAGRAM I. 5-carpelled flower with dextrorse spiral.
 DIAGRAM II. 5-carpelled flower with sinistrorse spiral.
 DIAGRAM III. 4-carpelled flower with dextrorse spiral.
 DIAGRAM IV. 3-carpelled flower with dextrorse spiral.

In measuring their carpels, each ovary was cut transversely and each placenta marked by touching the opened channel under it—between it and the ovary wall—with ink, on doing which a little of the ink passes up the channel and is seen from outside as a black streak. Thus marked, the lower half of the ovary was carefully 'centred' on a metal disk marked for the purpose with concentric

¹ Teratological observations on *Parnassia palustris*: Journ. Bot., 1896, pp. 12-15.

circles, and the distance from ink-mark to ink-mark, i. e. placenta to placenta, measured by means of a graduated and index-bearing scale which could be revolved round the metal disk. When the carpels had thus been measured, the relative position of the sepals was determined.

K. Schumann has described a similar machine in the *Berichte der Deutschen Botanischen Gesellschaft* (Bd. xi (1893), p. 248).

With my machine, 26 5-carpelled, 89 4-carpelled, and 20 3-carpelled fruits were measured. The diagrams on p. 187, showing the sepals and carpels, illustrate the results. In them the sepals are numbered in their sequence along the spiral—dextrorse or sinistrorse—and the carpels according to their size.

The largest carpel (No. I) stands over (in part at least) sepal No. 1; but carpel No. II—the second in size—has no relation to sepal No. 2, but is that to the side of carpel I—contrary to thread of spiral. Carpell III stands on the other side; IV and V, if present, are those more remote from I. In this way the fruit is seen to be made of carpels decreasing in size towards its late-developing side. In illustration of this are the following figures:—

TABLE I.

Size of carpels in 5-carpelled flowers with dextrorse spiral.

I.	II.	III.	IV.	V.
101°	89°	58°	70°	42°
97	75	74	69	45
96	70	73	74	47
91	82	80	59	48
90	69	90	62	49
88	78	67	66	61
86	60	71	80	63
85	62	70	88	55
84	89	75	64	48
76	65	68	77	74
72	84	75	71	58
<i>Total</i> 966	823	801	780	590
<i>Average</i> 87.8°	74.7	72.7	70.9	53.6

TABLE II.

Size of carpels in 5-carpelled flowers with sinistrorse spiral.

I.	II.	III.	IV.	V.
109°	58°	60°	84°	49°
108	67	76	57	52
107	72	79	75	27
102	76	75	66	41
100	71	69	69	51
98	71	71	72	48
98	76	81	60	45
97	76	79	60	48
97	81	58	58	66
91	81	81	63	44
89	71	82	73	45
86	69	75	80	50
86	86	68	77	43
86	74	76	70	54
71	78	76	66	69
Total 1425	1107	1106	1030	732
Average 95.0°	73.7°	73.7°	68.7°	48.8°

I did not make any measurements of the variation of the carpels from their *theoretical* superposition over the corresponding sepals.

The number of 4-carpelled fruits examined is too great to allow of the insertion of details in this note. Of the 89 examined, 52 were dextrorse, 37 sinistrorse. The average sizes obtained are as follows:—

TABLE III.

Average size of carpels in fruits with four carpels.

No. of Carpel.	I.	II.	III.	IV.
Dextrorse flowers (52)	105.1°	88.3°	83.3°	83.3°
Sinistrorse flowers (37)	99.4	88.5	87.0	85.0
Total (89)	102.7	88.3	84.9	84.0

In all but two of these 4-carpelled flowers the angle ACB was measured (see Fig. 1, Diagram III), the middle of sepal 4 being one fixed point, the near limit of carpel I being the other. It was found that in 84 cases the limit of the carpel extended beyond the middle of sepal 4; and in no case was carpel I superposed exactly to sepal I. The average result is indicated in Diagram III, where it is shown that the ultimate position of this carpel is over the petal the next in the direction of the spiral to sepal I. The average size of the angle ACB proved to be 14° ; the greatest angles measured were 61° , 29° , 28° , 27° , and so on; the least were -16° , -5° , and -2° , being the three cases in which the limit of the carpel did not extend beyond the middle of sepal 4.

It is not to be assumed that carpel I is always the largest. This is not so, as the following table indicates:—

TABLE IV.

Number of cases in which the various carpels were relatively large or small among 4-carpelled fruits.

<i>No. of Carpel.</i>	<i>Largest.</i>	<i>2nd.</i>	<i>3rd.</i>	<i>Least.</i>
I.	69	13	5	2
II.	13	37	28	11
III.	5	26	29	29
IV.	5	19	26	39

Turning now to 3-carpelled fruits, the following figures were obtained, and the following measurements of the angle BCD (see Diagram IV) which was measured instead of the angle ACB .

TABLE V.

Size of carpels in 3-carpelled flowers with dextrorse spiral.

I.	II.	III.	Angle BCD.
174°	86°	100°	+ 6°
148	110	102	- 13
136	120	104	+ 4
126	116	118	- 9
122	122	116	+ 6
121	129	110	- 46
120	136	104	- 3
113	140	117	- 17
<i>Total</i> 1060	959	861	- 72
<i>Average</i> 132.5°	119.9°	107.5°	- 9°

TABLE VI.

Size of carpels in 3-carpelled flowers with sinistrorse spiral.

I.	II.	III.	Angle BCD.
168°	87°	105°	+ 18°
168	87	105	- 7
168	80	112	+ 20
159	99	102	+ 11
151	101	108	+ 10
148	121	91	+ 18
135	118	107	- 17
131	126	103	- 8
127	135	98	- 29
126	125	109	- 26
124	135	101	- 29
105	138	117	+ 1
<i>Total</i> 1710	1352	1258	- 38
<i>Average</i> 142.5°	112.9°	104.8°	- 3°

The carpels are represented in their average proportion to each other, and their average position with regard to the sepals in Diagram IV. In this figure carpel I will be seen turned towards the direction of the spiral as in Diagram III, though not so markedly.

These observations may prove suggestive. I do not at present propose to make any deductions from them.

I. HENRY BURKILL.

THE PRIMITIVE ALGAE AND THE FLAGELLATA.—

Since the appearance of my article in the last number of the *Annals*, I have learned that the theory of the parallel evolution of the brown and green Algae, to the exposition of which a section of my article was devoted, was first suggested by Professor Wille so long ago as 1891. The hypothesis, and at that time it could be no more, was briefly brought forward in the chapters on Algae which Wille contributed to Warming's *Systematic Botany* (3rd Danish Edition, section *Syngeneticae*).

In the English translation of this work the editor has unfortunately omitted just the two sentences which contain this view, so that I was quite unaware that Wille had been the first to suggest it or I would gladly have associated with the theory the name of one to whom algology owes so much.

Further, I should like to add that on looking through the section on the phylogeny of the *Chlorophyceae*, it occurs to me that I might have dealt more directly with the views brought forward by Chodat at the British Association in 1896 (see *Annals of Botany*, vol. xi). These views have, however, their origin in conceptions of the polymorphism of the green Algae which cannot be said to be generally accepted, so that it would have been difficult to have considered them without introducing controversial details, but the spirit of the phylogenetic treatment is eminently scientific, and I am at one with the author in thinking that the broad lines of affinity are remarkably clear in this group.

Finally, I regret that the following reference to a paper which is mentioned in the text has been omitted from the bibliography:—West, G. S. '98, *Alga-Flora of Cambridgeshire*, *Journal of Botany*, vol. xxxvii.

F. F. BLACKMAN.

CAMBRIDGE.

The Development of the Pollen-tube and the Division of the Generative Nucleus in certain Species of Pines.

BY

MARGARET C. FERGUSON¹.



With Plates XII, XIII, and XIV.



INTRODUCTORY.

THERE is perhaps no phase of botanical science to which greater interest attaches at the present day than that which is concerned with the problems of sexual reproduction. The early botanists found in this question merely a favourite subject for philosophical speculation. Although Amici ('30-'46) made certain interesting observations regarding the development of the pollen-tube and the origin of the embryo in several plants, yet Hofmeister ('46-'62), whose works have already become classic, will ever be recognized as the first true scientific investigator along the line of sexual reproduction in plants. Since his studies, many botanists have found in this subject an attractive field for investigation. The celebrated discoveries of Ikeno, Hirase, and Webber, in 1897, gave a new incentive to this study, particularly in connexion with

¹ Read before the Botanical Society of America at its sixth annual meeting in New York City, June 28, 1900.

[Annals of Botany, Vol. XV. No. LVIII. June, 1901.]

the Gymnosperms, and rendered it highly desirable that fertilization and associated phenomena should be worked out for other members of this group by the more modern methods of investigation.

The present studies were undertaken in the fall of 1897. They have been carried on in the botanical laboratory of Cornell University under the direction of Professor George F. Atkinson. Professor Atkinson's interest in the work has been a constant inspiration to me, and his counsel invaluable. I also take pleasure in acknowledging my appreciation of the valuable assistance rendered by Dr. E. J. Durand.

METHODS.

On November 15, 1897, and each week thereafter until December 25, cones of *Pinus Strobus*, *P. austriaca*, *P. rigida*, and *P. montana*, var. *uncinata*, were collected, and ovules preserved. Material was fixed occasionally during the remainder of the winter. Beginning with April 1, the species named above and also *Pinus resinosa* were collected once each week. Collections were made twice each week during the month of May and three times a week for June. From June 10–30, a period which was sure to cover fertilization, cones of *Pinus Strobus* were collected every day at about nine o'clock in the morning, and frequently again at four in the afternoon. During May and June the little young cones were collected for each species as well as the more mature cones of the previous year's growth. After July 1, the older cones were no longer collected, but the young cones of *Pinus Strobus*, *P. austriaca*, and *P. rigida* were collected once each week until November 15. Cones of *Pinus Strobus* were again collected regularly, as described above, during the spring and early summer of 1899.

Each time of collecting, ovules were put up from several cones of each species, and these cones were not taken from the tip of one branch, but from different branches. Ovules were fixed from the central portion only of the cones. In the first stages of development the cones were fixed entire

or cut into quarters longitudinally. Very soon the individual scales were removed from the receptacle before fixing, and, when the scales were of sufficient size to admit of such manipulation, all superfluous parts were cut away, leaving the two tiny ovules still united by a small portion of the scale. In the spring the ovules were removed from the scales and, as soon as it was feasible, a portion of the integument was cut away from two or more sides of each ovule. For later stages the endosperms were frequently removed, but such material did not prove to be as satisfactory as that in which the nucellar cap and a small portion of the integument were left in connexion with the prothallium. Throughout the entire mechanical process of preparing material for the fixer, the most extreme care was used, as it was found that a very slight pressure was sufficient to cause distortions and thus render the material worthless for cytological studies.

The methods used in fixing and staining do not differ materially from those generally employed in cytological work. Several fixing fluids were tried, but the Flemming chromosmo-acetic acid solution gave by far the best results, and the other fixers were entirely discarded. The material was embedded in paraffin which had a melting point of 54° and was cut on a Minot-Zimmermann revolving microtome. Some sections were cut four, others thirteen and one-third, microns thick; but by far the greater part of the material was cut six and two-thirds microns thick.

Various stains were tried, among which might be mentioned Rosen's ('92) fuchsin and methylene-blue method; the Ehrlich-Biondi-Heidenhain mixture, as prepared by Dr. G. Grüber; Guignard's combination of methyl green, acid fuchsin, and orange G (Guignard has not given the formula which he uses and hence solutions of various strengths were tried); Flemming's safranin, gentian-violet and orange combination; and Heidenhain's iron-haematoxylin. The last two proved the most satisfactory. Flemming's triple stain was often used without the safranin with excellent results. The iron-haematoxylin was followed by orange G, or if it was desirable to

stain cell-walls, by Bismarck brown. Iron-haematoxylin, followed by Flemming's triple stain or by gentian-violet, and orange G, brought out the so-called kinoplasmic structures with great definiteness.

During the course of these investigations more than three thousand ovules have been sectioned, stained, and studied. The present paper is concerned only with the development of the pollen-tube; fertilization and associated phenomena will be considered in a second paper which is to appear subsequently.

HISTORICAL.

Comparatively few students have occupied themselves with the growth of the pollen-tube in the Abietineae, and no one, in so far as I have been able to determine, has described the cytological features attending the formation of the sperm-nuclei in this group.

In 1862 Hofmeister described and figured the pollen-grain in the Abietineae as consisting of a cell-complex, noted the depression at the apex of the nucellus in *Pinus* at the time of pollination, and traced the pollen-tube into the corpusculum. He also figured the pit in the apex of the pollen-tube which, however, he said remained closed until after the formation of the proembryo, when it was ruptured by mechanical means. Hofmeister further observed what were doubtless the sperm-nuclei at the apex of the pollen-tube, but he did not understand their function and was unable to determine their later history.

The works of Strasburger on this subject have been more numerous and complete than those of any other investigator. It is extremely interesting to note how his interpretations have kept pace with the improvements in methods of research. In 1869 he followed the pollen-tube into the corpusculum in *Pinus* and *Picea* and confirmed Hofmeister's observation regarding the presence of a closed pit at the apex of the pollen-tube; but he did not observe the nuclei in the pollen-tube, and remarked that inasmuch as the sexual organs touch

in these plants, spermatozoids would be superfluous and are, in reality, not present. He added, however, that their place is taken by granular protoplasm and starch-grains which exercise the same fertilizing effect on the egg as do spermatozoids. In 1872 Strasburger detected two cells in the pollen-tube of several Gymnosperms, but considered that such cells were extremely rare in the Abietineae, as he had only once found one in this group. The shrunken remains of these cells were seen in the pollen-tube after fertilization. He thought that the pit of the pollen-tube remained closed, and that the exchange-substance was apparently communicated by a vacuole between the apex of the pollen-tube and the egg-nucleus. Six years later ('78) he observed two nuclei in the pollen-tube of *Pinus* and *Picea* when the tube was just above the archegonium. According to his interpretation at that time, the nucleus in front was dissolved while the one behind entered the egg and fused with its nucleus. This was a great advance on his previous observations; but he still conceived of the pollen-tube as remaining closed, and fancied that the protoplasmic contents passed through the membrane directly, while the starch was dissolved before its transmission into the egg. In the following year ('79) he established the fact that it is the foremost of the two sperm-nuclei in the pollen-tube which becomes active in fertilization.

Goroschankin ('83) saw the two sperm-nuclei pass into the egg in *Pinus Pumilio*, and he believed that both fused with its nucleus. Strasburger ('84) confirmed his observation as to the passage of the two sperm-nuclei from the pollen-tube into the egg, but pointed out that only the one in advance fuses with the egg nucleus.

It was left for Belajeff ('91) to establish the true nature of the cell-complex found in the pollen-grain of the Gymnosperms. He demonstrated the fact that in *Taxus baccata* the large nucleus of the pollen-grain is the vegetative or pollen-tube nucleus, as in the Angiosperms, and that the sperm-nuclei arise by the division of one of the smaller cells of the pollen-grain. This smaller cell divides to form the stalk- and the

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generative cell. The latter cell passes into the pollen-tube before giving rise to the sperm-nuclei.

Strasburger ('92) showed that Belajeff's observations on the development of the pollen-tube in *Taxus baccata* were in general true for the other Gymnosperms. He described the mature pollen-grain in *Pinus sylvestris*, noted that the vegetative nucleus passes into the pollen-tube immediately upon germination, and remarked that the last formed prothallial cell remains in its place in the pollen-grain until the following spring, when it divides into the stalk- and the body-cell of the antheridium. The division of this cell was not studied, but Strasburger thought it took place at about the same time as the development of the archegonia. He also stated that the lower sperm-cell is the larger, and described each cell as being almost entirely filled with its large, coarsely granular nucleus.

Belajeff ('93) worked out the development of the pollen-tube in *Picea* as a type of the Abietineae. He found that the generative cell divides while still in the pollen-grain, and gives rise to two sperm-cells which he figured as of the same size.

Dixon ('94) traced the history of the pollen-grain and the pollen-tube in *Pinus sylvestris* from the time of pollination to fertilization. According to his observations *Pinus* agrees in the main with *Picea*, as described by Belajeff. In general I cannot confirm Dixon's results, and they will be considered more fully in the body of this paper.

In giving an account of some work done by his students on the Gymnosperms, Coulter ('97) reported that the work of Dixon 'was largely confirmed in the minutest detail,' and in 1900 he figured the pollen-tube 'in pines,' when just above the archegonium, showing two sperm-cells of equal size.

Blackman ('98) did not study the growth of the pollen-tube, but accepted Dixon's work as authoritative.

Professor Atkinson ('98) stated that the sperm-mother-cell in *Pinus* divides into two sperm-cells *after* having passed into the pollen-tube.

POLLINATION.

In the vicinity of Ithaca pollination takes place in the pines during the latter part of May or the first week in June, the different species varying a few days. When the pollen-grains have been drawn into the depression in the upper part of the nucellus, the projecting portions of the integument close over them and a resinous substance is secreted which practically seals the opening by which the pollen-grains entered (Plate XII, Figs. 1 and 7). At this time the pollen-grain contains, as described by Strasburger ('92), the remnants of two prothallial cells; one small cell, the third prothallial cell formed, or the antheridial cell; and a large cell, the vegetative or wall-cell (Fig. 6).

The degree of development which the ovule has attained at the time of pollination is shown in Fig. 1. The central portion of the nucellus is occupied by an axial row of cells, the lowest of which is in the early prophase of division (the figure is drawn on too small a scale to show this distinctly); and the two upper nuclei are in a process of disintegration, as indicated by their diffuse reaction to stains. Frequently only the first cell of the axial row, the macrospore of Hofmeister, is present at this time. The so-called spongy or loose tissue of Strasburger is already well differentiated when pollination takes place.

The upper concave portion of the nucellus which, together with the enveloping integument, forms the pollen-chamber, terminates in a row of more or less elongated cells which are not closely united at their free extremities, but stand out, as it were, like so many fingers to catch the pollen-grains; they also serve to facilitate the entrance of the pollen-tube into the tissue of the nucellus (Fig. 7).

DEVELOPMENT OF THE POLLEN-TUBE.

The germination of the pollen-grain takes place very soon after pollination. The pollen-grain increases slightly in size, the concave portion of the wall (*d*, Fig. 6) becomes convex,

then bulges out, the exospore is ruptured, and the endospore is gradually prolonged into a tube. Immediately upon the formation of the pollen-tube, the vegetative nucleus, as shown by Strasburger ('92), moves away from the antheridial cell and towards the tip of the pollen-tube (Figs. 7 and 8). Strasburger ('92) describes the antheridial cell in *Pinus sylvestris* as remaining unchanged until the archegonia are formed in the following spring. Dixon states that it divides about a month before fertilization, but from a careful reading of the text, one is given the impression that this was an inference on his part rather than a demonstrated fact. In some species of pines the division of this cell certainly takes place very much earlier. During the first week in August there have been found on the nucellus, in *Pinus Strobus*, pollen-grains containing two cells (Fig. 10). In such instances the vegetative nucleus can invariably be detected in the pollen-tube. In the same material, other pollen-grains are observed in which the division of the antheridial cell has not yet taken place; but in material fixed somewhat later, it is only rarely found undivided. In *Pinus austriaca* this division also takes place during the summer. I once found the mitotic figure for the division of the antheridial cell in *Pinus resinosa* on the first of April. As this was the earliest date on which material of this species had been collected, I am unable to determine at present whether or not it is the normal time for the division in *Pinus resinosa*.

A pollen-grain with its antheridial cell still undivided has been observed just prior to fertilization (Fig. 5). In this case two of the three pollen-tubes which have almost reached the prothallium are furnished with sperm- and stalk-cells, while in the third, only the vegetative nucleus is found. On the apex of the nucellus there is a pollen-grain which at this late date contains one cell, the antheridial cell, still undivided. The nucleus of this pollen-grain (Fig. 5 *b*) is large, plump, and to all appearances perfectly normal, and it is possible, though scarcely probable, that it may still divide. That one cannot trace a definite connexion between the pollen-tube containing

only the vegetative nucleus and this pollen-grain signifies little, for those who have studied the pollen-tube of *Pinus* know that it is the exception rather than the rule when a given pollen-tube can be traced through the lacerated dead tissue of the upper portion of the nucellus to the pollen-grain from which it proceeded. Such a condition as that just described is rarely met with at so late a date. But occasionally during the summer and fall pollen-grains are found in which no cell-division has taken place since pollination, although in the majority of cases the stalk- and the generative cell have already been formed. These observations seem to indicate that, while the division of the antheridial cell takes place comparatively soon after the pollen-grain has germinated in *Pinus Strobus* and *P. austriaca* (other species have not been studied with regard to this point), it may under certain conditions be much delayed, and in some cases, doubtless, never takes place at all. This question can only be decided by actual observation of the mitotic figure at different periods of the year. Meanwhile, we know that in *Pinus Strobus* and *P. austriaca* at least, the stalk- and the generative cell are formed as a rule before the approach of winter. At first these two cells are very similar, but the generative cell soon increases somewhat in size (Figs. 10 and 11).

A vertical section of an ovule collected on Jan. 4 is represented in Fig. 2. The spongy tissue surrounds a cavity crossed by irregular strands of protoplasm in which the free nuclei of the prothallium are embedded. The stalk and the generative cell are enclosed within the pollen-grain, and the vegetative nucleus is near the apex of the irregularly branched pollen-tube. This pollen-tube is shown more highly magnified in Fig. 12. At this time the pollen-tubes have penetrated the nucellus almost to the point at which it joins the free limb of the integument. The greatest depth to which the tubes may have grown is not indicated in the illustration. The section was figured because it shows most clearly the cells of the pollen-grain and tube. Other sections

of the same ovule would have shown pollen-tubes which had pierced to a greater depth into the nucellus. The conditions of development as figured for January coincide perfectly with those which exist during the latter part of October.

Growth is very slow during the first period of development, but with the renewed activities of spring the ovule increases rapidly in size; the central cavity of the nucellus becomes greatly enlarged and is lined with the growing endosperm. The prothallium now consists of a uniform layer of protoplasm in which numerous free nuclei are embedded, no cell-walls as yet having been laid down. Immediately surrounding the endosperm, there is a definite band or hollow sphere of cells which is limited on its outer surface by a thin stratum of the disintegrating nucellus. These two layers constitute the so-called spongy tissue. The inner portion of this tissue, i.e. the prominent band in immediate contact with the prothallium, must be intimately connected with the nutrition of the young endosperm. The true structure and function of this layer seem to have escaped the notice of previous writers. Its cells contain large nuclei, and are abundantly supplied with protoplasm. The karyokinetic figures so frequently observed in these cells show that this tissue increases in size by the growth and division of its cells, as do the other portions of the ovule. As it enlarges, the cells of the nucellus in contact with its outer surface become disorganized and are absorbed (Fig. 3).

The cells of the nucellar cap which were penetrated by the pollen-tubes during the previous season do not again become active, but remain as deeply staining, thick-walled, dead cells. The cells just beneath them, however, multiply rapidly, and become literally packed with starch. A few of the cells from this region in Fig. 5 are shown more highly magnified in Fig. 18. By the growth and increase of these cells, the dead top of the nucellus with its pollen-tubes is lifted far above the developing endosperm, so that the pollen-tubes, once so near their goal, are now removed from it by a considerable distance (Figs. 2-4). Meanwhile the pollen-

tube has increased little, if at all, in length, renewed activity in the male gametophyte being first indicated by a further development of the cells still within the pollen-grain.

The stalk-cell increases in size and its cytoplasm assumes a vacuolate character. The growth of the generative cell is still more marked, and its cytoplasm on the contrary becomes dense and deeply staining. (Compare Fig. 12, Jan. 4, with Fig. 13, May 3.) In *Pinus sylvestris*, as studied by Dixon, the generative cell divides while it is within the pollen-grain. In the species of pines which I have investigated, this division does not occur until the generative and the stalk-cell have entered the pollen-tube, and the stalk-cell has passed below the generative cell. As the generative cell increases in size it stretches out towards and into the neck of the pollen-tube, drawing after it the stalk-cell, or possibly being forced out by this cell, the two passing into the tube together. Dixon states that only the naked nucleus of the stalk-cell enters the pollen-tube, and in so far as I am aware, no writer has described the entrance of the entire stalk-cell into the pollen-tube in the Abietineae. The material which I have studied shows conclusively that the nucleus does not 'slip out' of its cytoplasm (Figs. 12-15). The entire cell can be identified in the tube and later in the egg. During the time that this cell is moving over the generative cell its cytoplasm cannot always be clearly differentiated from that of the latter; but when once the stalk-cell has passed the generative cell, its nucleus surrounded by a sphere of very vacuolate cytoplasm, scarcely more than a peripheral layer, is again distinctly demonstrated. After passing the generative nucleus, the stalk-cell ordinarily takes up a position between the generative cell and the vegetative nucleus (Plate XIII, Fig. 21); occasionally it may pass the vegetative nucleus (Fig. 22).

When the generative cell leaves the pollen-grain, its nucleus is situated near the top of the cell, but it apparently moves faster than does the accompanying cytoplasm, and when the stalk-cell passes the generative nucleus, this nucleus lies at or below the centre of its cell (Figs. 13, 19, and 20). Shortly

after this the generative nucleus is again observed at the uppermost part of its cytoplasm (Figs. 21 and 22).

During its passage into the tube, the generative cell increases much in size; it has no definite cell-wall, and its cytoplasm forms a large, irregular tongue about the nucleus. This cytoplasm in no way suggests the alveolar structure of Bütschli ('94), but is distinctly reticular, differing in appearance from the nuclear net only by its greater delicacy. This is shown more clearly at a somewhat later stage.

The vegetative and generative nuclei are now very similar in structure, though each is sufficiently characteristic to be readily recognized by one who is familiar with them. The vegetative nucleus has one large, usually homogeneously staining nucleolus, rarely one or more smaller nucleoli, and it is furnished with a rather scanty, delicate reticulum which is apparently poor in chromatin. Either it is in a state of more or less collapse, or it is very hard to fix at this period, for its outline is as a rule quite irregular. The generative nucleus has one large, hollow or vacuolate nucleolus, and commonly two smaller ones; its reticulum, though more abundant than that of the vegetative nucleus, is still delicate and often shows a weak reaction to nuclear stains. The stalk-nucleus has a very decided individuality which it maintains throughout its entire history. It bears a strong resemblance from the first to the nuclei of the nucellar tissue, rarely, if ever, contains a true nucleolus, and its close-meshed reticulum is conspicuous for its comparatively large net-knots or karyosomes.

DIVISION OF THE GENERATIVE NUCLEUS.

Dixon ('95) describes this division in *Pinus sylvestris* as taking place about a *month before fertilization*, while the generative cell is still *within the pollen-grain*; and Coulter ('97) states, as already mentioned, that in his study of *Pinus Laricio* he has been able to confirm Dixon's observations. At this time, as pointed out by Dixon, the nuclear and cytological phenomena are very greatly obscured by the

presence in the pollen-tube of large quantities of starch (Fig. 20). This starch, which resists the microtome knife and is therefore easily displaced by it, not infrequently falls out and carries away with it the free cells of the pollen-tube. The dead, deeply staining tissue of the nucellus, representing that portion of the nucellar cap which was penetrated by the pollen-tube during the previous season, and in which the generative cell divides (Fig. 4), is also very troublesome. Furthermore the dense cytoplasm of the generative cell shows a great affinity for stains, so that when the archegonia and other portions of the ovule are well stained, this cell often appears merely as a deeply stained mass of no significance. Considering these facts, it is not surprising that seven hundred slides of serial sections were made, which means that more than two thousand pollen-tubes were studied, before any definite clue was obtained as to the true sequence of events in the development of the pollen-tube. When once the mitotic figure was observed *in the pollen-tube*, scarcely more than a *week before fertilization*, and the fact noted that special staining was necessary in order to study this mitosis satisfactorily, further research was prosecuted with comparative ease.

After the generative cell has passed into the pollen-grain, but while it is still in the upper dead portion of the nucellus, it gives rise to the sperm-nuclei by a division which presents some new and interesting features, while it resembles to a greater or less degree certain mitoses described by various cytologists¹ during the past few years.

When the generative nucleus has again come to lie in

¹ Of the long list which might be mentioned I have noted only the following: Guignard ('91) in the embryo-sac of *Lilium*; Rosen ('95) in the root-tip of Hyacinth; Osterhout ('97) in *Equisetum*; Swingle ('97) Sphacelariaceae; Schaffner ('98) in root-tip of *Allium Cepa*; Mottier ('98) in the embryo-sac of *Lilium*; Fulmer ('98) in pine seedlings; Hof ('98) in *Ephedra* and other plants; Nemec ('98 and '99) in various plants; Strasburger ('00) in *Vicia Faba*, and Mottier ('00) in *Dictyota*. Of animal-cytologists I will mention but one: Hertwig, R., ('98) in *Actinosphaerium*. This division in *Actinosphaerium* bears in certain stages of its prophase a most striking resemblance to an early prophase of the mitosis about to be described.

the extreme upper portion of its cell, certain changes in the cytoplasm indicate that division is being initiated. At some little distance below the nucleus the cytoplasm shows a finely granular structure which is not at this stage dense nor deeply staining. From this region irregular granular threads arise which extend outward towards the periphery of the cell, those extending in the direction of the nucleus forming a hollow cone over its lower portion (Fig. 23). Gradually the granular area increases in density and in staining capacity, at the same time drawing nearer to the nucleus which is separated from it by a hyaline court. Into this court delicate granular threads pass (Fig. 24). When these threads reach the nuclear membrane, the nucleus is forced so close against the peripheral layer of cytoplasm that its wall is frequently indented on the upper side, while the condensation from which the so-called kinoplasmic threads arise withdraws, or is forced by the growth of the threads, further from the nucleus. A great number of delicately anastomosing threads now extend, in the form of a solid cone, from a point within the granular condensation up towards and against the nucleus. The outer threads of the cone pass over the lower portion of the nucleus and appear in sections of the cell as closely packed against either side of the nucleus. At the same time, the entire cytoplasmic reticulum has assumed a more or less radial arrangement, into which some of the more delicate threads extend (Fig. 25).

Co-ordinately with these changes in the cytoplasm, the chromatin of the nuclear net collects in spherical or irregular masses on the reticulum, and sooner or later gives rise to a broad spirem, along which the chromatic disks are distributed at regular intervals (Figs. 23-26). Whether the formation of this spirem precedes or follows the penetration into the nuclear cavity of the achromatic threads seems to depend upon the length to which these threads attain. They may become very long when their entrance into the nucleus is delayed; but more frequently a portion of the nuclear membrane gives way, and some of the achromatic fibres pass into

the nuclear cavity before the spirem is established (Figs. 26 and 29). Rarely, the nuclear membrane appears pushed in irregularly along its entire lower margin, as indicated in Figs. 25 and 26; as a rule, however, there seems to be one deep, sharp indentation along one side of which the nuclear wall first gives way (Figs. 27 and 29). With the initial steps in the disappearance of the nuclear membrane the nucleolus is either not apparent or, if still demonstrable, it stains but feebly. When the membrane disappears along the entire lower portion of the nucleus, the kinoplasmic threads press so closely against it that it cannot be definitely demonstrated whether it passes into the cytoplasmic and the nuclear reticulum or becomes fibrous and contributes to the formation of the achromatic threads (Figs. 28 *b* and 29). The threads which have been packed so closely against the wall of the nucleus now press into the nuclear cavity and mingle with those which have entered from below; and the dense, granular, cytoplasmic area from which the threads diverge is gradually dissipated (Fig. 30).

In the early stages of mitosis it is evident that there are present in the nuclear reticulum two kinds of granules, as described by Heidenhain ('93 and '94). This becomes more apparent as the chromatin condenses to form the spirem. When the spirem is fully differentiated there is present in the nuclear cavity a loose, granular, achromatic reticulum. With the disappearance of the wall along the lower part of the nucleus this network seems to undergo a partial rearrangement. A portion of it is resolved into threads of more or less regularity, which in general assume a position parallel to the threads entering the nuclear cavity; some of them become attached directly to the ends of these fibres, and doubtless contribute to their growth (Figs. 29-31).

As the spindle-threads proceed in their development across the nucleus, the chromatic spirem collects in the region of the future nuclear plate, and becomes more or less massed together, presenting an appearance somewhat comparable with that of synapsis; at the same time it becomes homogeneous

and gives rise by segmentation to the chromosomes (Figs. 30-33). Some of the ingrowing spindle-threads may extend across the nucleus to the nuclear membrane, which is still present on the upper side of the nucleus; but by far the greater number unite some distance below this membrane to form several poles, thus giving rise to a diarch spindle (Strasburger, 1900), which is multipolar at its upper extremity; and unipolar, or nearly so, at its lower end. Gradually the poles of the upper portion draw together, while the spindle is somewhat shortened by the lower extremity of the threads being again resolved into granules. Finally a true bipolar diarch spindle is formed with the V-shaped chromosomes oriented at the nuclear plate. Each pole terminates in a slight granular condensation. The upper pole has never been observed to reach to the nuclear membrane, but frequently coarse granular threads extend from the pole to the wall of the nucleus, and apparently act as supports (Fig. 34). These are doubtless formed by a rearrangement of the linin reticulum. The nuclear membrane persists along the upper part of the nucleus until the late teleophase of the division (Pl. XIII, Figs. 30-35, and Pl. XIV, Fig. 38).

As the chromosomes pass to the poles, the central spindle elongates so that the daughter-nuclei are separated, as a rule, by a greater distance than the original length of the spindle. While this is characteristic of cell-division in general, it is occasionally much exaggerated here, the daughter-nuclei being apparently forced apart with considerable energy. The nucleus which occupies the position nearest to the micropylar end of the ovule usually shows a deep indentation along its upper surface as if a resistance had been met with in the peripheral layer of cytoplasm (Figs. 41 and 42). Not infrequently the upper nucleus is found almost entirely separated from the cytoplasm (Fig. 43). This, however, may be due to mechanical rupture during sectioning and staining. No cell-wall is ever formed, and in only one instance was a condensation of the spindle-threads in the region of the cell-plate observed (Fig. 40). The spindle may become

contracted at or near its centre during its disintegration, thus presenting the appearance of an hour-glass, or it may give rise to such a condition as that shown in Fig. 42. This figure with various modifications is not uncommon. I am unable to trace its origin definitely, but it is not improbable that it is caused by a contraction of the cytoplasm resulting from a cessation of the force which tends to separate the daughter-nuclei; or it may be caused by the resistance which the peripheral layer of cytoplasm, along the outer surface of the upper nucleus, offers to the growing fibres, thereby forcing them back upon themselves as shown in the figure. When all traces of the spindle have disappeared, the two sperm-nuclei are surrounded by a common mass of cytoplasm.

The mitosis just described seems to be unique as regards the origin and development of the achromatic spindle. The most exaggerated instance of asymmetry in spindle-formation which I have found recorded is that described and figured by Nemec ('99, 2) in *Solanum tuberosum*. Here the nucleus lies at one side of the cell, and the spindle-fibres are very much more prominent on the free side of the nucleus than on the side adjacent to the cell-wall. In another paper Nemec ('99, 3) shows by experimentation that the form of the figure which gives rise to the extranuclear spindle depends upon external forces or conditions. In the case of the pines studied, the position of the generative nucleus is such that the spindle if extranuclear in origin must of necessity be unipolar, since there is no cytoplasm, or almost none, above the nucleus from which fibres could arise.

The blending of the linin reticulum with the cytoplasmic network after the disappearance of the lower portion of the nuclear membrane, and the relation of certain portions of the achromatic nuclear reticulum to the ingrowing fibres, suggest an intimate relation between these structures. That the spindle-fibres which originate in the cytoplasm and apparently grow by a differentiation of its network are later fed by the linin of the achromatic nuclear reticulum there seems little room for doubt. In fact, all the phenomena connected with

this division indicate that we are dealing, not with persistent cell-constituents, but with different manifestations of one and the same thing. In a word, we find no evidence here of the presence in the cell of a definite kinoplasmic substance. Farmer and Williams ('98) in a study of *Fucus* 'do not regard the kinoplasm as a persistent protoplasmic structure, but as forming the visible expression of a certain phase of protoplasmic activity.' Wilson ('99 and '00) states that the astral rays 'grow by a progressive differentiation out of the general cytoplasmic meshwork,' and he finds in the Echinoderm's egg 'no ground for a specific kinoplasm.'

Nothing has been said regarding the nature of the granular, cytoplasmic condensation from which the achromatic spindle takes its origin. It never has a definite boundary, though it is often very clearly differentiated by its dense granular appearance and its strong affinity for stains; but it may be inconspicuous or fail entirely to be demonstrated. So much has been said during the past decade regarding the nature and existence of the centrosome and the centrosphere that one feels inclined to avoid the subject entirely. Yet the question may very properly be asked, Is this condensation which forms the centre of a system of radiating fibres a centrosphere? It certainly is as clearly an attraction-sphere as some figures which have been described as such; but if we accept Wilson's ('00) definition of the centrosphere, the body under consideration cannot be so denominated, as no centrosome has been observed at its centre. More deeply staining granules may sometimes be present within the condensation, but these are not considered of any significance, as such granules may be found anywhere in the cytoplasm.

Karsten ('93) describes the nucleoli in *Psilotum* as passing out of the nucleus and assuming the role of centrosomes, and Strasburger ('00) considers that the nucleoli not only contribute material for the formation of kinoplasmic threads, but that they also make active the spindle-forming substance in the cytoplasm—in other words, they act as the kinetic centres of the cell. There seems to be no evidence that such is the

case here, for the nucleoli, after the condensation has arisen and the spindle-threads have attained considerable length, are morphologically the same as they were before the inception of the spindle. Nemec ('99, 1) remarks that in the higher plants where the centrosome is not present, the entire nucleus may exercise the function of the centrosome. The idea of the diffused centrosome in the cells of the higher plants was suggested by Guignard ('97), and again hinted at by Le Dantec ('99). If we may accept Guignard's suggestion, then the kinetic centre of the cell in the higher plants is no longer indicated by the presence of a definite organ, the centrosome, but the power of this organ has become dissipated throughout the entire cell. When that phase of cell-activity which has to do with spindle-formation comes into play, the points at which it is centred would naturally be indicated by a greater accumulation of the microsomes, and thus an aster of more or less definiteness would be formed, as when the individualized centrosome is present. In the division under consideration, the position of the generative nucleus is such that the energy active in spindle-formation must perforce be centred at some point below it. Such a centring of activity would naturally result in an attraction-sphere of unusual prominence; and there would be no occasion for its division since the spindle is unipolar in origin.

The endosperm has become a solid mass of tissue at the time when the generative nucleus divides. The details of its development will not be given here, more than to say that it resembles in many points the growth of the prothallium in *Taxus baccata* as described by Jäger ('99). The archegonia are still comparatively small and quite vacuolate and the central cell has not yet divided (Fig. 4).

GROWTH OF THE SPERM-NUCLEI AND LATER HISTORY OF THE POLLEN-TUBE.

After the mitotic figure has entirely disappeared, the sperm-nuclei are separated by a considerable distance; and the form which the cytoplasm surrounding them assumes seems to

vary with the shape of the pollen-tube. Gradually the two nuclei approach each other until they come to lie in the extreme uppermost part of their cytoplasm (Figs. 47 and 48). There is now considerable difference in their size. This inequality in size could be detected as far back as the formation of the daughter-nuclei (Figs. 39 and 40). Coulter ('97) described two sperm-cells which were of the same size until within the archegonium. Blackman ('98) stated that each sperm-nucleus was clearly seen in the pollen-tube surrounded by its own cytoplasm, but he did not figure them. Chamberlain ('99) figured the sperm-nuclei, in *Pinus Laricio*, of equal size in the pollen-tube, and showed them lying together in the cytoplasm of the tube. Not having seen these cells within the archegonium before the conjugation of the sexual nuclei, he accepted Coulter's statement for the growth of one of them after their entrance into the egg. Two sperm-cells have not been observed in any of the pines which I have studied; but the sperm-nuclei, which are of unequal size from a very early date, remain surrounded by a common cytoplasmic body (Figs. 39-50). As Strasburger ('92) observed, the larger nucleus is always ahead, that is, on the side nearest the apex of the pollen-tube. The smaller nucleus remains close against the upper boundary of the cytoplasm and suggests the condition in *Cycas* (Ikeno, '98) and *Ginkgo* (Hirase, '98) where the stalk-nucleus is forced entirely out of the cytoplasm surrounding the generative nucleus. In this case the action is not carried to so great an extent.

Only once has the smaller sperm-nucleus been observed in advance of the larger (Fig. 44). Here it will be seen that the entire order of arrangement has been changed and the stalk-cell and the vegetative nucleus are above the sperm-nuclei. But this abnormal arrangement is only apparent, for it was found that the egg which had been approached by this pollen-tube had already been fertilized, and the pollen-tube had turned aside and was passing up over the top of the endosperm. The position of the various elements of the pollen-tube is therefore normal, and the larger sperm-cell is in reality in advance of the smaller.

The formation of the sperm-nuclei shows very beautifully the development of the nuclear reticulum. The chromosomes unite end to end, giving rise to a homogeneous, coiled band, before the nuclear membrane is formed. When the nuclear wall has been differentiated, the coil expands about the periphery of the nucleus and the band broadens, at the same time becoming irregularly jagged along its margins. These irregularities increase in length until finally those from neighbouring portions of the thread meet and fuse, thus giving rise to the reticulum (Figs. 37-40).

When the two sperm-nuclei have nearly or quite come into contact they have as a rule reached their mature size. More than a year has now elapsed since pollination. Up to this time the pollen-tube has elongated very slowly, having penetrated as yet little beyond the nucellar tissue of the previous year's growth. In this upper portion of the nucellar cap the tube may become very broad or it may branch freely (Figs. 3, 4, 12, and 16). When the sperm-nuclei have attained their full size the downward growth of the tube is exceedingly rapid, as shown by Dixon, and the tube is unbranched and comparatively straight (Fig. 5). In *Pinus Strobus* and *P. austriaca* about ten days intervene between the division of the generative nucleus and fertilization; in *Pinus montana*, var. *uncinata*, the two processes are separated by an even shorter space of time.

The sperm-nuclei which at first present a very beautiful, rather delicate reticulum (Figs. 47 and 48), become more dense as the pollen-tube advances through the nucellus. Strasburger ('92) describes them as coarsely granular, but, with a high power, the presence of a reticulum which is sometimes coarse and interrupted can invariably be made out in well-prepared material. By the time that these nuclei have reached the central portion of the nucellar cap they have usually become very dense in structure (Figs. 46 and 46 b), and frequently stain intensely, though they may show at this time a weak reaction to dyes. The reticula of the two nuclei may present the same appearance, or they may differ as in

the figures above referred to. The nucleolus, if it be present at this time, is usually obscured by the dense network. Arnoldi ('00) describes the sperm-nuclei in *Cephalotaxus* as being gradually filled with metaplast. I see no evidence of such a process in the development of these nuclei in *Pinus*.

When the pollen-tube reaches the egg, its apex is abundantly supplied with cytoplasm, in the upper part of which the vegetative nucleus lies. The sperm-nuclei are just above, with the stalk-cell still in contact with the lower portion of their cytoplasm. Still higher up, the tube may contain many starch grains (Fig. 50). There is never any doubt at this time as to the identity of the stalk-cell and the vegetative nucleus in the material which I have studied; but Dixon states that they cannot be distinguished, and Coulter ('97) describes them as having lost their original outline.

Archoplasmic areas similar to those figured by Chamberlain ('99) have been observed in connexion with the sperm-nuclei, but as such granular accumulations may occur at any point in the cytoplasm no importance is attached to them.

Chamberlain ('97) describes a multiplication of the normal number of cells in the pollen-grain of *Lilium*; and Arnoldi ('00), finding more than the usual number of nuclei in the pollen-tube of *Cephalotaxus*, considers that more than one vegetative or wall nucleus has been formed. I have twice observed such an excess of nuclei in *Pinus*. Three nuclei have been found in the pollen-grain after the vegetative nucleus has passed into the pollen-tube (Fig. 51), and two nuclei have been seen just passing into the pollen-tube while the stalk-nucleus could still be detected in the pollen-grain, though it was almost obscured by the dead nucellar tissue and is not shown in the sketch (Fig. 45). It is not possible to determine definitely from either of these preparations to what portion of the male gametophyte the extra cells belong. Two wall-cells may have been formed or there may be present two stalk-cells; I am inclined to believe that the former is true in Fig. 45 and the latter in Fig. 51, but in neither case can one affirm positively, and there is a possibility

that in both instances two generative nuclei have been formed.

When Professor Atkinson mentioned the pines as a favourable subject for investigation, he referred to the then recent discoveries of Ikeno, Hirase, and Webber, and remarked that it would be most interesting to determine whether any suggestions or remnants of a cilia-forming body (called blepharoplast by Webber in *Zamia*) still persist in the Conifers. Somewhat later, after the present research was begun, MacMillan ('98) pointed out the desirability of such a study both in Coniferae and Gnetales. I have seen no indication in connexion with the formation of the sperm-nuclei in *Pinus* of a structure which might be regarded as a reduced blepharoplast or as suggestive of a cilia-forming body of any sort. Inasmuch as spermatozoids do not exist here, such an organ, if present, must be functionless. But the cytoplasmic radiations which accompany the division, in its early stages, of the generative nucleus (Figs. 25 and 36), seem to differ in degree only from those found by Webber ('97, 1) in the generative cell of *Zamia*, as shown in his Figs. 3 and 5; and the question may be raised whether in this cytoplasmic figure we may not have still persisting in the cell the last vestiges of such an organ as that described by Webber. Neither has anything been observed throughout this study to indicate that the sperm-nuclei of *Pinus* ever assume the spiral or reniform shape, suggestive of spermatozoids, which has been described by recent writers¹ for the sperm-nuclei in various Phanerogams. The nuclei early become spherical or elliptical in outline, depending on the breadth of the pollen-tube, and remain so during their entire later history.

SUMMARY.

1. The structure of the pollen-grain agrees fully with that described by Strasburger ('92).
2. Pollination takes place in the neighbourhood of Cornell

¹ Golinski ('93) in certain grasses, Nawaschin ('99), Guignard ('99) and Sargent ('99) in *Lilium*, Merrell ('00) in *Silphium*, and Thomas ('00) in *Caltha*.

University, $42\frac{1}{2}^{\circ}$ North lat., during the latter part of May or early in June.

3. The pollen-grain germinates very soon after pollination, and the vegetative nucleus immediately passes into the tube.

4. The division of the antheridial cell takes place in *Pinus Strobus* and *P. austriaca* before the beginning of winter. It is probable that this cell does not always divide at a definite time, but that in a given species the time during which it may divide extends over a considerable period.

5. During the first season the pollen-tube grows very slowly, and it may be broad and irregular in outline or it may branch freely.

6. Shortly before fertilization the generative cell, followed by the stalk-cell, moves into the pollen-tube. The stalk-cell soon passes the generative cell and takes up a position near the vegetative nucleus. These changes and those immediately following are frequently much obscured by the presence in the pollen-tube of large quantities of starch.

7. The generative cell, as the other cells of the pollen-grain, is never limited by a well-defined cell-wall, and consists at the time of its division of an irregular protoplasmic body, in the upper part of which the nucleus lies.

8. In the division of the generative nucleus the spindle is extranuclear and unipolar in origin.

The formation of the spindle indicates that the cytoplasmic network and the nuclear reticulum have essentially the same structure, and the spindle-fibres are apparently formed by a transformation of both.

The nuclear membrane persists along the upper part of the nucleus until the early stages in the formation of the daughter-nuclei.

This division takes place a little more than a year after pollination and from a week to ten days before fertilization, about thirteen months elapsing between pollination and fertilization.

9. Two sperm-cells are never formed, but the sperm-nuclei remain surrounded by a common mass of cytoplasm. An

inequality in the size of these nuclei is very early apparent, and becomes more pronounced as they reach maturity.

10. The sperm-nuclei soon come to lie together in the upper part of their cytoplasm and early attain their full size, the larger one being invariably in advance.

The nuclear reticulum, at first delicate, soon becomes very dense, but there is no evidence of the presence in these nuclei of a special metaplasmic substance.

11. At the time when the sperm-nuclei come into contact or nearly so, the pollen-tube has penetrated little, if at all, beyond the nucellar tissue of the first year's growth. Now, however, it again begins to elongate, and its downward course through the new nucellar tissue is extremely rapid.

12. When just above the egg, the apex of the pollen-tube is filled with cytoplasm. The vegetative nucleus lies in the upper part of this cytoplasm, and near it is seen the stalk-cell still in contact with the lower portion of the cytoplasm which surrounds the sperm-nuclei.

13. No individualized centrosome has been observed; but the existence of the diffused centrosome is suggested in connexion with the division of the generative nucleus.

14. The above summary holds good, when not otherwise indicated, for all five species of pines which I have studied. Nuclear phenomena are found to vary so much, even within the limits of a given genus, that it no longer seems safe to consider the details of development in a single plant as typical of a large group of plants. We therefore make no generalizations regarding the Abietineae. And we hesitate even to draw conclusions for the genus *Pinus*, for while the agreement in certain phases of development of five species would seem to be sufficient for the formulation of a rule, there may still exist within the genus individuals which are, in certain aspects of nuclear activity, a law unto themselves.

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EXPLANATION OF FIGURES IN PLATES XII, XIII, AND XIV.

Illustrating Miss Ferguson's paper on the Pollen-tube of Pines.

All figures were drawn by the aid of the Abbé camera lucida, the image being thrown down only 143 mm. A Zeiss microscope was used and (with the exception of Figs. 1-5 and 44) the 2 mm. homogeneous immersion objective. This objective was combined with the compensating ocular No. 8 for Figs. 23-43, 46, and 46 b; for all other figures the compensating ocular No. 4 was used. The figures are reproduced from the drawings without reduction. Throughout the plates the lettering is to be interpreted as follows: third prothallial or antheridial cell (*a. c.*), vegetative nucleus (*v. n.*), stalk-cell (*st. c.*), stalk-nucleus (*st. n.*), generative cell (*g. c.*), generative nucleus (*g. n.*), sperm-cell (*s. c.*), sperm-nucleus (*s. n.*), prothallium (*Pr.*), spongy tissue (*s. t.*), archegonium (*arch.*), and starch grains (*s. g.*).

PLATE XII.

Fig. 1. A vertical section through an ovule some days after pollination; *A. r.*, axial row; *p. g.*, pollen-grain. $\times 70$. *Pinus Strobus*, June 17.

Fig. 2. A vertical section of an ovule showing the winter condition. $\times 70$. *Pinus Strobus*, Jan. 4.

Fig. 3. A vertical section of an ovule soon after the second period of growth has begun. $\times 70$. *Pinus Strobus*, May 26.

Fig. 4. A vertical section through the upper part of an ovule at the time of the division of the generative nucleus; *n. c. 1*, that portion of the nucellar cap which was developed during the first period of activity; *n. c. 2*, that part of the nucellar cap which constitutes the second year's growth; *o*, disintegrating spongy tissue. $\times 70$. *Pinus Strobus*, June 9.

Fig. 5. A vertical section through the upper part of an ovule shortly before fertilization; *e. n.*, egg nucleus; *o*, last vestige of spongy tissue. $\times 70$. *Pinus Strobus*, June 15.

Fig. 5 b. Pollen-grain from the nucellus of Fig. 5. The antheridial cell is still undivided. $\times 540$.

Fig. 6. Mature pollen-grain; *p.¹*, first prothallial cell; *p.²*, second prothallial cell. $\times 540$. *Pinus Strobus*, June 8.

Fig. 7. A vertical section through the extreme upper portion of an ovule soon after pollination, showing the uppermost part of the nucellar cap, and a pollen-grain in the first stages of germination; *P. c.*, pollen-chamber. $\times 540$. *Pinus Strobus*, June 13.

Fig. 8. A pollen-grain soon after germination; *p.²*, second prothallial cell. $\times 540$. *Pinus Strobus*, June 24.

Fig. 9. A pollen-grain after the vegetative nucleus has passed into the pollen-tube. $\times 540$. *Pinus Strobus*, July 15.

Fig. 10. A pollen-grain after the antheridial cell has divided. $\times 540$. *Pinus Strobus*, Aug. 4.

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Fig. 11. The same at a later date, showing a slight increase in the size of generative cell. $\times 540$. *Pinus Strobus*, Oct. 7.

Fig. 12. The pollen-tube which is shown in Fig. 2, more highly magnified. $\times 540$. *Pinus Strobus*, Jan. 4.

Fig. 13. A pollen-grain and the upper portion of a pollen-tube, showing the stalk- and the generative cell just before their passage into the pollen-tube. $\times 540$. *Pinus austriaca*, May 3.

Figs. 14 and 15. Later stages than the above, showing the passage of the generative and the stalk-cell into the pollen-tube; in Fig. 15 the two cells are breaking loose from each other. $\times 540$. *Pinus austriaca*, May 10 and 17.

Fig. 16. The male gametophyte at the time of the entrance into the tube of the generative and the stalk-cell; *n. t.*, a bit of the dead nucellar tissue. $\times 540$. *Pinus Strobus*, June 9.

Fig. 17. A pollen-grain after the generative and the stalk-cell have passed into the pollen-tube; taken from the top of the nucellus of Fig. 4. $\times 540$. *Pinus Strobus*, June 9.

Fig. 18. A few of the cells from that portion of the nucellar cap marked *n. c. 2* in Fig. 4. The cells are filled with starch-grains. $\times 540$. *Pinus Strobus*, June 9.

PLATE XIII.

Figs. 19–21. Portions of pollen-tubes showing successive stages in the passage of the stalk-cell over the generative cell, as also the presence of large quantities of starch in the pollen-tube. $\times 540$. *Pinus resinosa*, June 2; *P. Strobus*, May 24; *P. rigida*, June 8.

Fig. 22. The generative cell, bearing on its surface both the vegetative and stalk-nuclei. In this instance the stalk-cell has passed beyond the vegetative nucleus. $\times 540$. *Pinus resinosa*, June 3.

Figs. 23–25. The generative cell in the early stages of its division, showing granular condensation and radial arrangement of cytoplasm. $\times 850$. *Pinus rigida*, June 8 and 10.

Fig. 26. A later stage of the same. $\times 850$. *Pinus austriaca*, June 10.

Fig. 27. The generative cell just before the disappearance of the lower portion of the nuclear membrane. $\times 850$. *Pinus Strobus*, June 9.

Figs. 28 and 28 *b*. Two sections through the same generative cell. In Fig. 28 the section was cut through the edge of the nucleus and shows the spindle-fibres passing over the outside of it. Fig. 28 *b* represents a section nearer the middle of the nucleus: the nuclear wall has given way at the centre and is fading out along the entire lower portion of the nucleus; some spindle-fibres are entering the nuclear cavity, while others stretch along the surface of the disintegrating membrane. Neither section was cut parallel to the major axis of the growing spindle. $\times 850$ *Pinus austriaca*, June 10.

Fig. 29. A stage in spindle-formation directly following that shown in Fig. 27. $\times 850$. *Pinus Strobus*, June 10.

Figs. 30–35. Later stages in the development of the spindle, showing the gradual drawing together of the outer extremities of the threads to form the upper pole of the spindle, as also the origin of the chromosomes. $\times 850$. Fig. 33, *Pinus rigida*, June 13; the other figures, *Pinus austriaca*, June 7–10.

Fig. 36. A cross-section through the generative cell during an early stage in its

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mitosis. The protoplasmic condensation is seen from below looking toward the nucleus. $\times 850$. *Pinus austriaca*, June 4.

Fig. 37. The sperm-nuclei just after their formation. $\times 850$. *Pinus montana*, var. *uncinata*, May 31.

PLATE XIV.

Figs. 38-43. Various stages in the formation of the daughter-nuclei. $\times 850$. Figs. 38 and 43, *P. Strobus*, June 9 and 10; Fig. 39, *P. resinosa*, June 15; Figs. 40 and 42, *P. austriaca*, June 10. Fig. 41 represents another section through the upper nucleus of Fig. 40, and shows how the upper of the sperm-nuclei is frequently indented along its outer surface.

Fig. 44. This pollen-tube, having approached an egg that had already been fertilized, has turned aside and is passing up over the endosperm so that the normal position of the cells appears exactly reversed; *n. a.*, neck-cells of the archegonium. $\times 330$. *Pinus Strobus*, June 20.

Fig. 45. The generative cell and another nucleus, not the stalk-nucleus, just passing into the pollen-tube. $\times 540$. *Pinus Strobus*, May 20.

Figs. 46 and 46 *b*. Cross-sections through the two sperm-nuclei after they have attained full size and have about reached, in their downward passage, the middle of the nucellar cap. $\times 850$. *Pinus Strobus*, June 15.

Fig. 47. The sperm-cells after all traces of the spindle have disappeared, but before the two nuclei have come together. $\times 540$. *Pinus Strobus*, June 13.

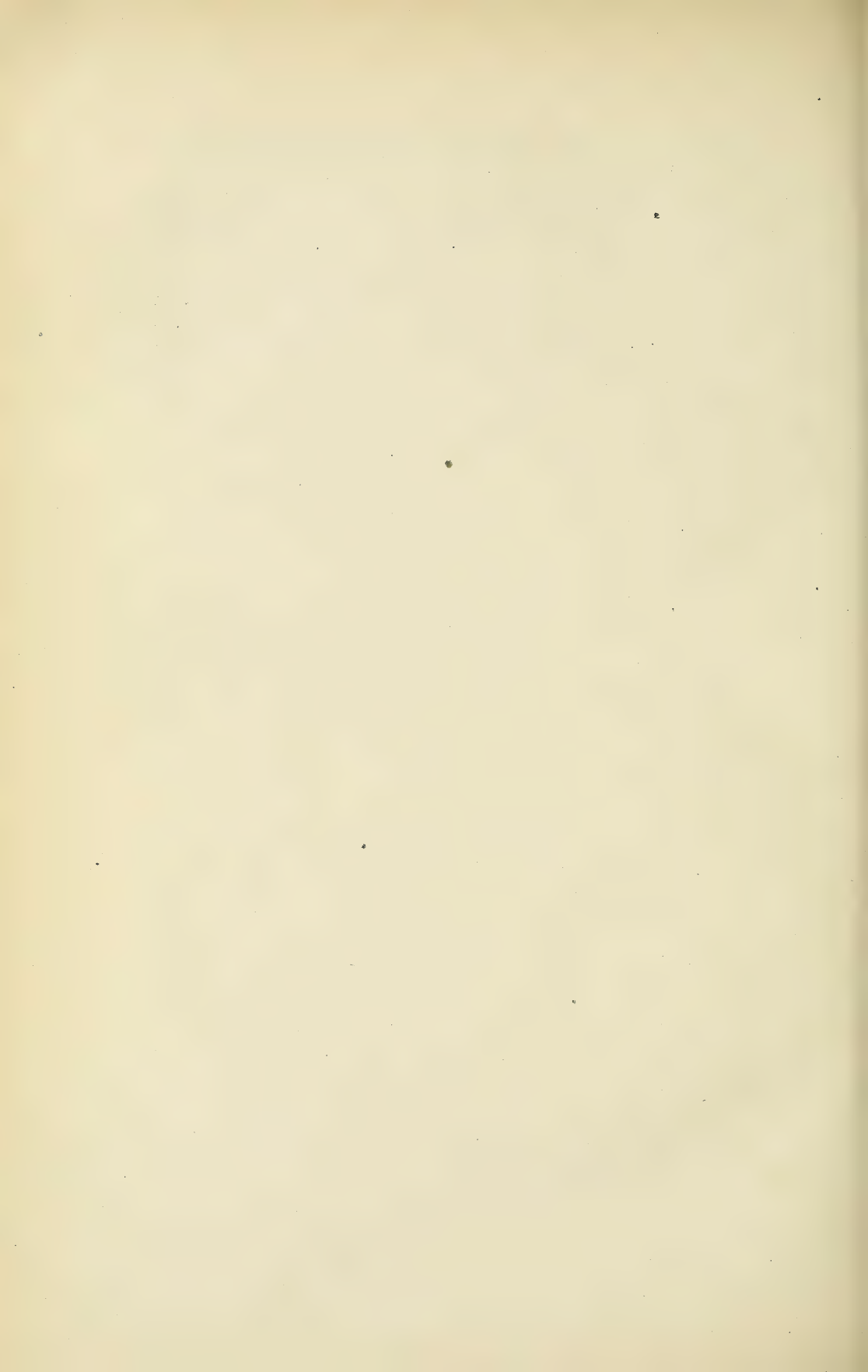
Fig. 48. The same after both nuclei have come to lie in the upper part of their cytoplasm. $\times 540$. *Pinus Strobus*, June 10.

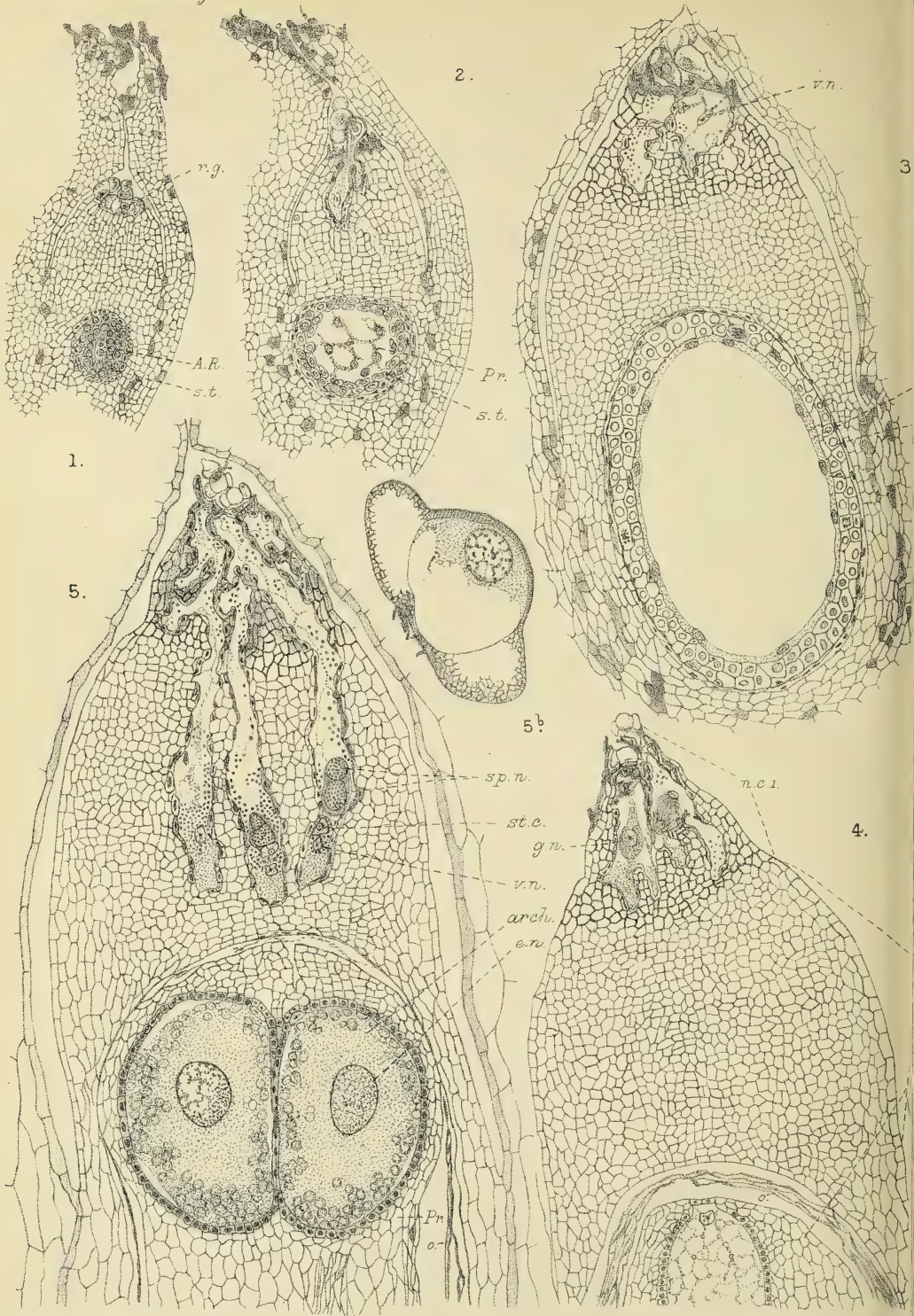
Fig. 49. Lower portion of a pollen-tube which has penetrated about two-thirds the length of the nucellar cap. $\times 540$. *Pinus Strobus*, June 14.

Fig. 50. Lower portion of a pollen-tube which is just pushing between the neck-cells of the archegonium. *P*, pit in apex of tube. $\times 540$. *Pinus Strobus*, June 20.

Fig. 51. A pollen-grain showing an increase in the normal number of nuclei. $\times 540$. *Pinus austriaca*, May 17.

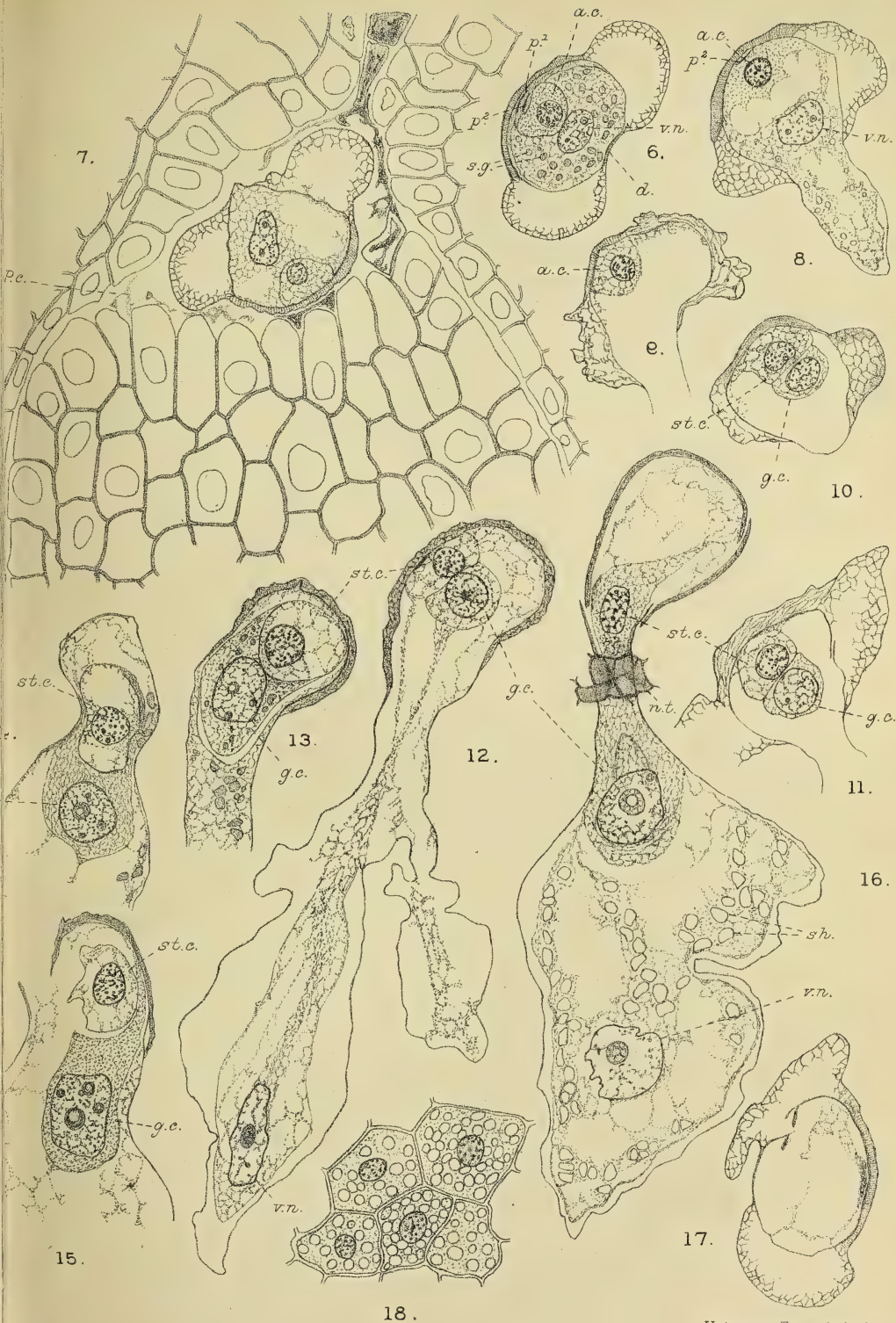
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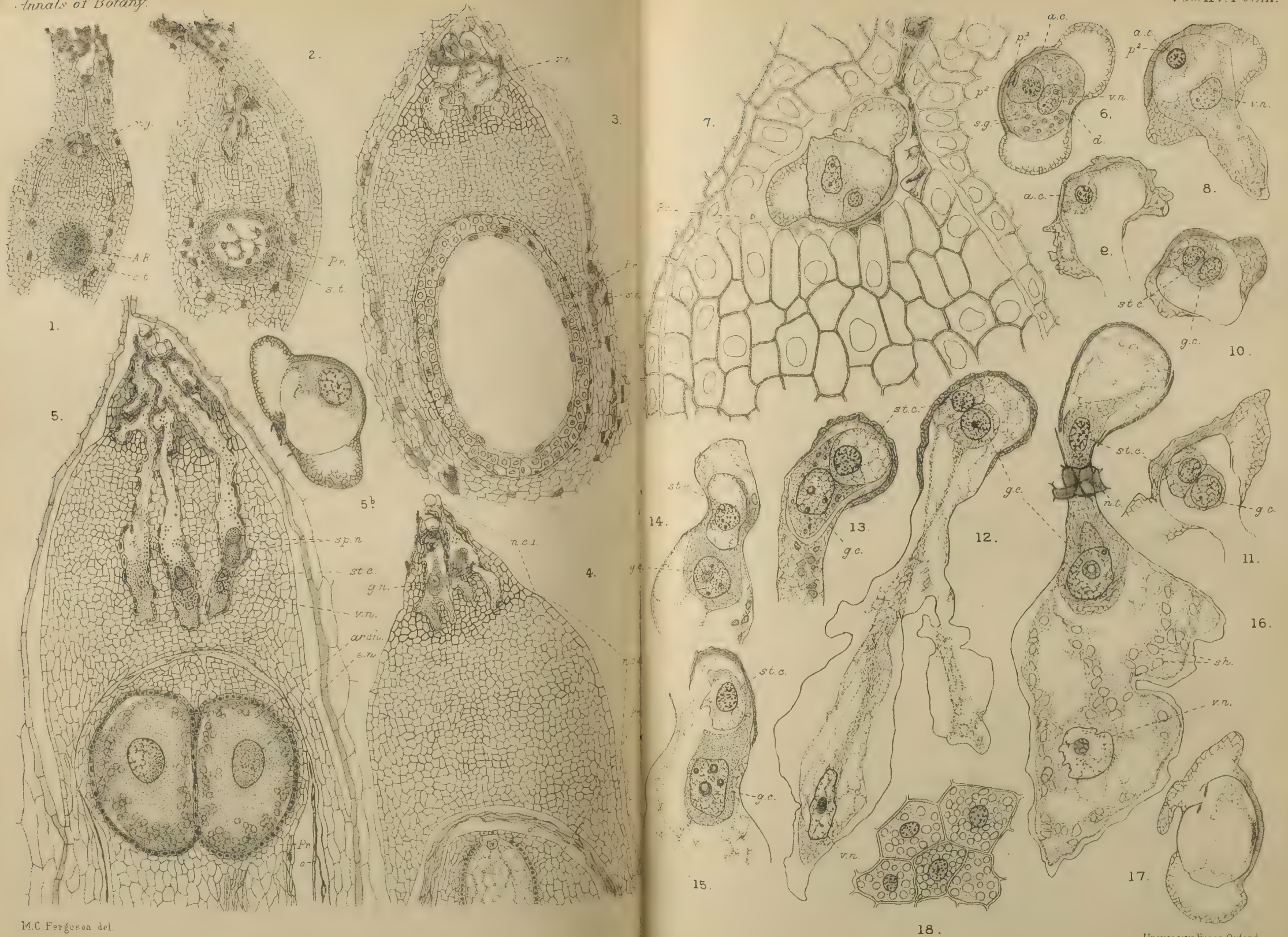




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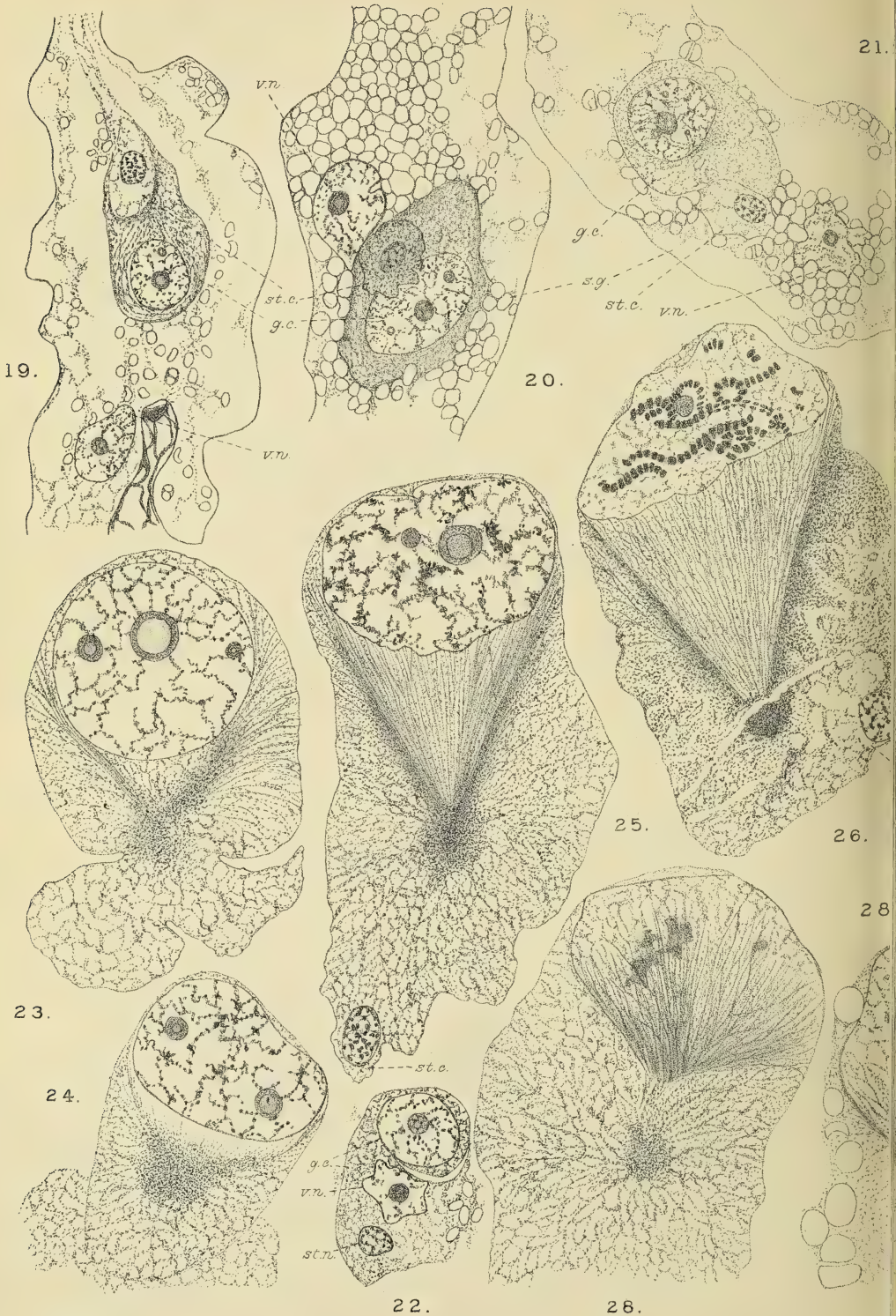




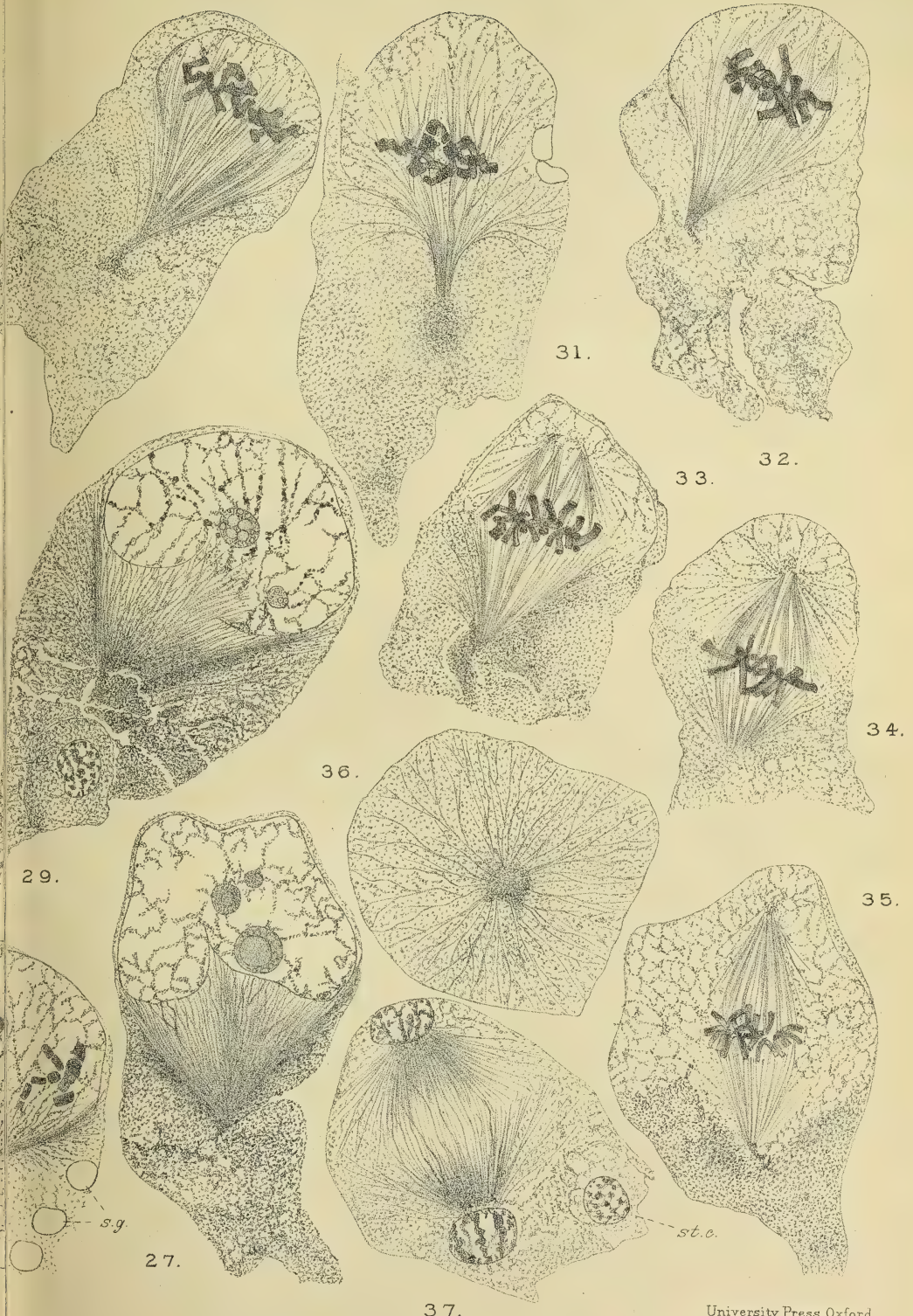
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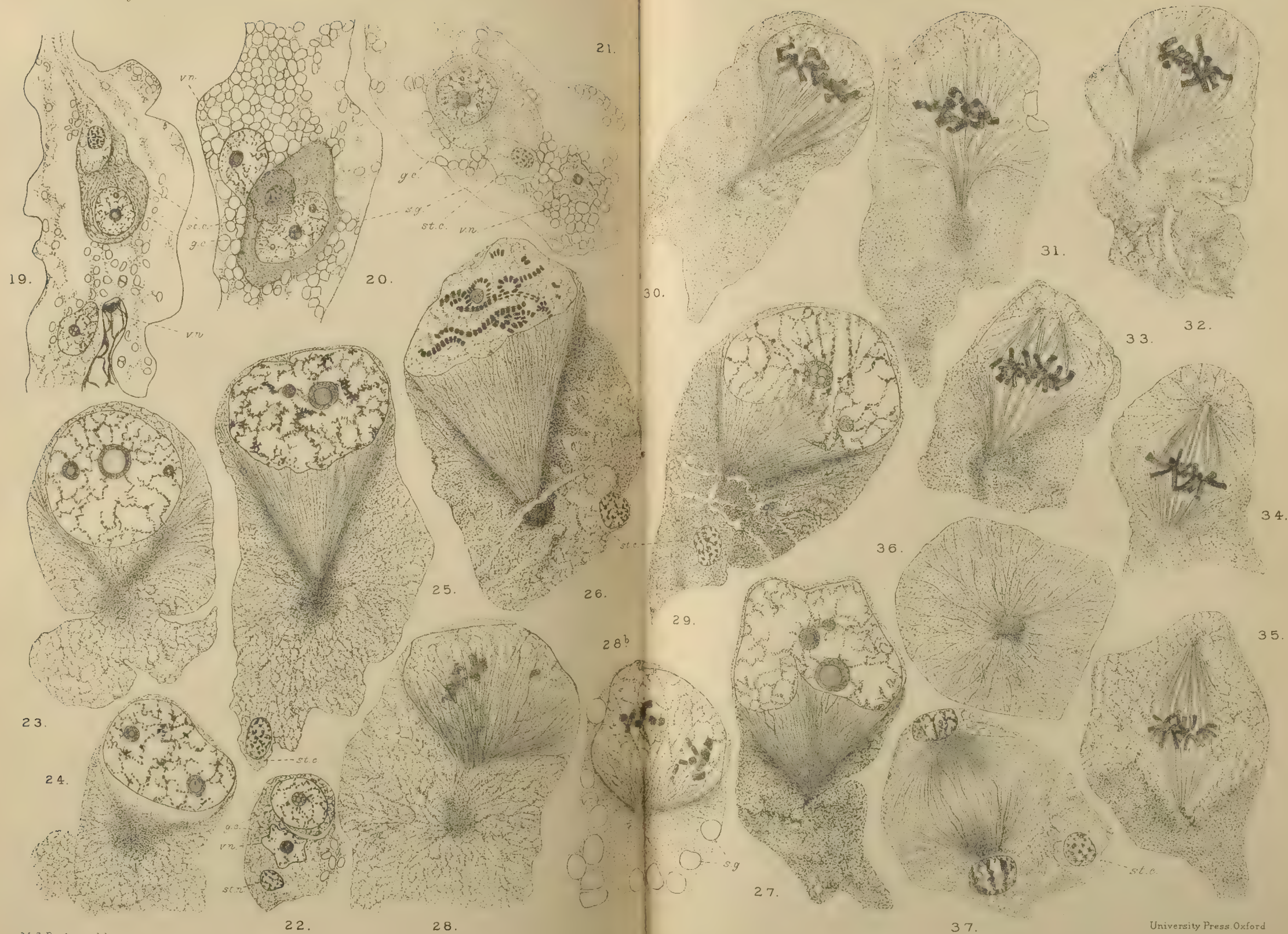
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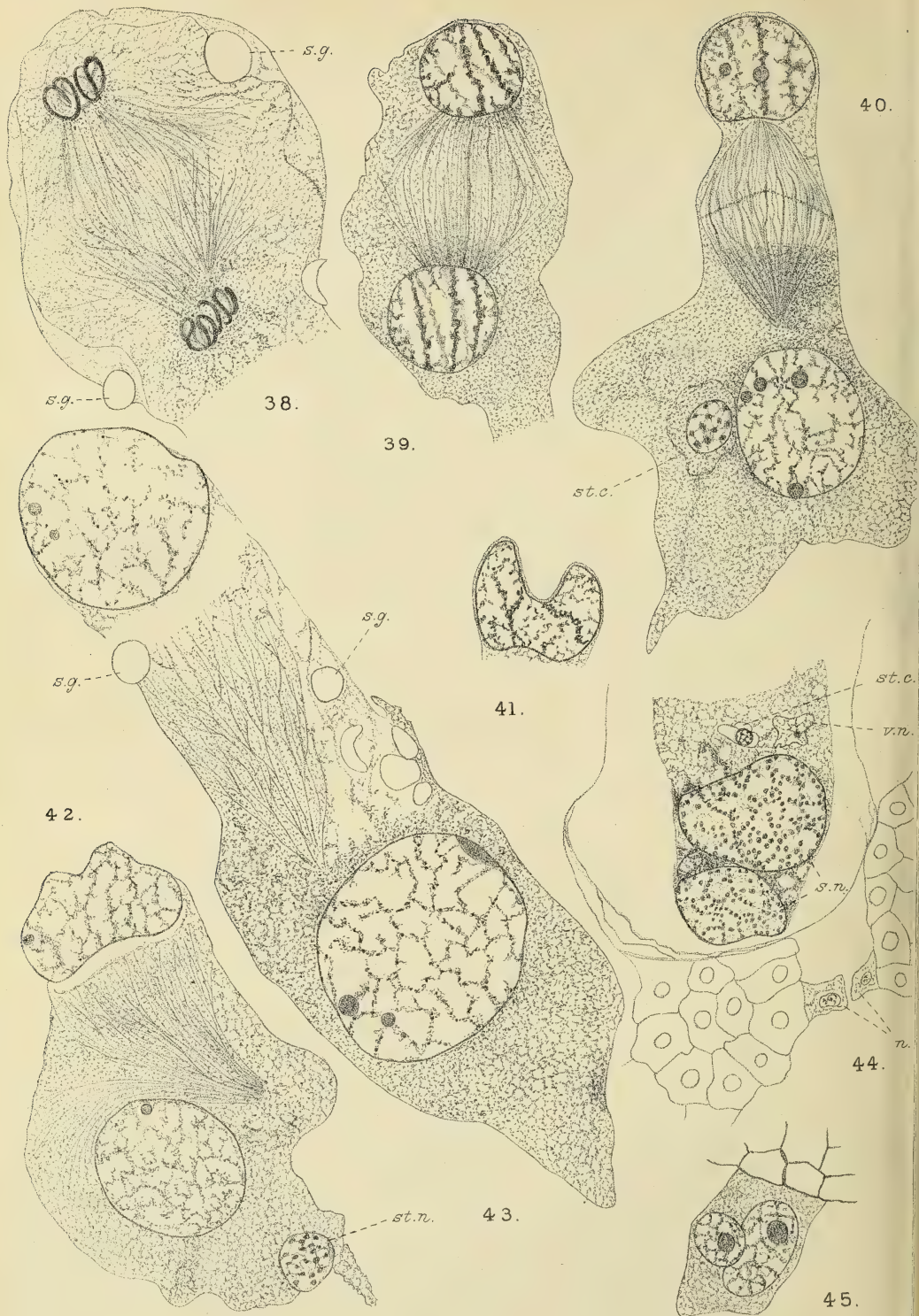


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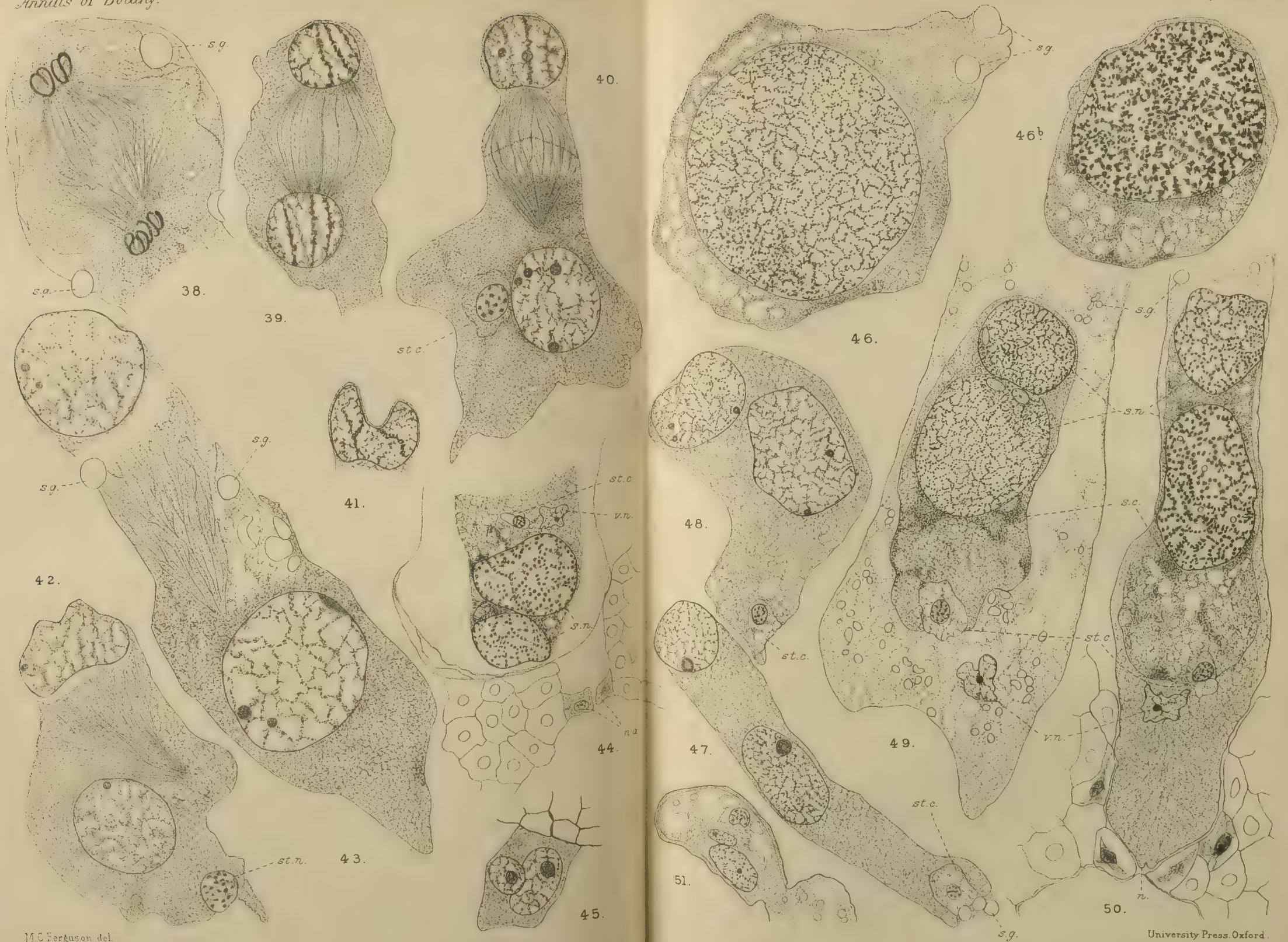
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Imperfect Sporangia in certain Pteridophytes. Are they vestigial?

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IMPERFECTLY developed parts have played an important rôle in arguments on Evolution. On the zoological side especially they have been used as weighty evidence. Similarly, on the botanical side they have been the basis of discussion: in the morphology of the flower, abortive stamens, carpels, pollen-sacs, and ovules have been cited as foundations for elaborate comparative argument. For instance, where present in normal-position the existence of an abortive stamen, or staminode, has been accepted as sufficient indication of the previous existence of a fully developed stamen in the ancestral line; and on such evidence natural affinities have been traced and accepted, usually without question.

But floral morphology has gone further: comparative study has led to the conclusion that in certain ancestral lines of descent parts have existed, which in the descendants of the present day are entirely unrepresented by any vestigial growth. This condition of complete disappearance of a part or parts has been styled 'ablast,' as distinct from 'abortion,' where the incompletely developed part has an objective existence¹. Against the former, as a fiction of Comparative

¹ These distinctive terms were introduced by Schmitz (Hanstein's Bot. Abhandl., Bd. ii, Heft 1, p. 57).

Morphology, Wigand raised an anti-evolutionary protest¹, maintaining that an essential difference exists between the two, and that 'ablast' exists only in the imagination, as a consequence of the assumption of unity of type in large groups of plants. It is doubtless salutary that such protests should be made, and they should have the effect of checking the exuberance of those who would extend a 'Type-method' beyond due limits, in the study of living forms. Fortunately there is now less need for such warnings than in the early seventies, the present tendency being towards broader phylogenetic views.

At the time of its issue Wigand's protest was suitably met by Eichler, who maintained that the conditions distinguished as 'abort' and 'ablast' are not essentially different in kind, but only differ in degree. He points out that abortion itself is not susceptible of objective proof: 'objectively we see nothing more than that cell-divisions occur, that a rudiment appears; thus strictly speaking we observe that something develops, not that something is reduced. This may become a gland, an emergence, or what not. It is comparison, and usually the comparison with other species and genera, &c.—that is, the type-method—which teaches that it is a reduced organ, and what is its special category. Whenever the same comparative method leads even to the assumption of a complete suppression, where no rudiment of the organ is seen with the bodily eye, in my opinion that is, in point of fact, no more than one step further along the same course².' This is the position which, within suitable limits, is at the foundation of the current view as to abortion of parts within the Angiospermic flower, and it is extended also to the vegetative organs.

The discussion above quoted related in the first place to parts such as stamens and carpels. But the same arguments are also applicable to individual sporangia. As an example,

¹ Darwinismus, i, p. 444. 'Der Abortus nach der Typen-Methode und nach der Descendenz-Theorie.'

² Eichler, Blüthendiagramme, p. 52.

the abortive ovules in the carpels of the Clematideae may be mentioned; though in the mature state only one ovule is found fully developed in each carpel, several are initiated in position corresponding to those of the Helleboreae; on these facts the argument may be based that a plurality of ovules was more widely the rule in the ancestors of the Ranunculaceae than it is at the present day¹. A similar case as regards the pollen-sacs is found in the stamens of *Salvia*. There is thus no reason to restrict the method to axes, leaves, and so forth, but it may be, and has been, applied to sporangia equally with other parts.

In the Pteridophytes, too little attention has hitherto been paid to such subjects, and notably observations of arrest of the spore-producing parts have been neglected. It is the comparative isolation of many of the genera, and the paucity of species in some of the most important of them, which has stood in the way of arguments from arrest taking their proper place in the morphology of the Pteridophyta. But the argument to be based on an imperfect sporangium of *Lycopodium*, or the abortive fertile spike of an *Ophioglossum*, seated in the position normal in other individuals, species, or allied genera, for a fully matured one, is precisely the same as that on an imperfect stamen or carpel, pollen-sac, or ovule. Further, a comparison as regards the presence or absence of spore-producing parts, in species evidently related to one another, may lead to the conclusion that sporangia, entirely unrepresented at the present day, were probably present in the ancestry; the line of argument being the same as that in the cases of hypothetical 'ablast,' or complete suppression of floral parts.

In this paper I propose to bring together certain cases of incompletely developed sporangia, or spore-producing parts in the Pteridophyta, in order that those facts may have their due weight in the general discussion of the balance of the vegetative and fertile regions of the primitive leafy sporo-

¹ Eichler, Blüthendiagramme, p. 174; also Prantl, Engler's Jahrbücher, vol. ix, p. 237.

phyte. The cases quoted are chiefly from among the Club-Mosses, in which the problem is more readily handled than in most other Pteridophyta.

PHYLLOGLOSSUM.

I have already drawn attention to the fact that the transition from the protophylls of this plant to the sporophylls is usually abrupt¹. Occasionally the last of the protophylls to be formed is smaller than the rest, and thus it approaches in size to the normal sporophyll. But Mettenius² states that sometimes the fertile shaft bears a sterile bract some small distance below the strobilus. In my own specimens, in one case, a sporophyll of larger size than usual, with a sporangium attached, was found isolated below the strobilus³: thus there are indications of a transition between the protophylls and the sporophylls. In the strobilus itself all the lower sporophylls bear sporangia; Bertrand remarks, however⁴, that though this is the case for the three lowest whorls of the spike, the sporangia on the fourth whorl are not always fully developed, those of the fifth whorl are atrophied, while those of the sixth bear but vague traces of sporangia: such sporangia may appear only as slight convexities of the surface, while still higher all traces may be absent. Mettenius also noted that the sporophylls at the apex of the strobilus may be sterile.

I have seen such abortive sporangia in the apical region of the strobilus of *Phylloglossum*; I doubt, however, any numerical constancy of those fully formed, for I have seen sections of a strobilus in which the leaves of the fourth whorl were without any trace of a sporangium.

It is thus seen that while there is in *Phylloglossum* as yet no detailed evidence of abortion of sporangia at the base of the strobilus, or in connexion with the protophylls, still occasionally there are intermediate types between the proto-

¹ Studies, i, p. 506.

² Bot. Zeit., 1867, p. 99.

³ Loc. cit., Pl. xliii, Fig. 22.

⁴ Archives Bot. du Nord, vol. ii, pp. 83, 127.

phylls and the sporophylls. But abortion, partial or complete, does take place at the apex. It will be a matter for subsequent discussion what is the comparative bearing of these facts.

LYCOPODIUM.

This genus was divided by Spring¹ according to the distribution of the sporangia, an arrangement which is maintained, with amendments, by Baker in his *Fern-Allies*². In dealing with the abortion of sporangia, and their distribution upon the plant, I shall follow Baker's arrangement, giving, in the order of the species in his work, the details which I have thought worthy of note for the present purpose, after looking through the herbaria of Kew, and of Glasgow University.

By adopting for the present purpose Baker's arrangement, I do not deny that it is open to such amendments as have been proposed elsewhere³.

Sub-genus. *Selago*.

Group of *L. Selago*.

1. *L. compactum*, Hook. Old sporangia were observed at the leaf-bases, even down to the base of large plants. How early does this fertility begin in the individual?

3. *L. Selago*, L. There is usually a sterile region at the base of the plant: this is followed by the well-known alternating fertile and sterile zones. About the limits of these zones, sporangia of smaller size are found, which sometimes remain closed when all those in the neighbourhood have dehisced. There is no marked change of size or form of leaf, on passing from the sterile to the fertile zones. Occasionally a single sporangium may be found in an otherwise sterile region.

6. *L. erythraeum*, Spring. A long sterile basal region is found in some specimens.

¹ Monographie des Lycopodiacees, p. 18.

² Handbook of the Fern-Allies, 1887, p. 8.

³ Engler and Prantl, Pflanzenfamilien, i, 4, p. 591.

7. *L. Saururus*, Lam. There is usually a sterile basal region of some length; no obvious external distinction of sterile and fertile regions.

9. *L. Hamiltonii*, Spreng. (incl. *L. vernicosum*, H. & G.). Plants from Khasia (C. B. Clarke) show transition from sterile to fertile region very gradually as regards leaf-form; at most there is a gradual and ultimate diminution of size. Occasional single sporangia may be found far down in the sterile region, in the axil of leaves of the larger sterile type. This has been seen in many other specimens besides Clarke's Khasia plants.

11. *L. reflexum*, Lam. As in *L. Hamiltonii*, isolated sporangia were found in the lower sterile region.

12. *L. miniatum*, Spring. The same.

13. *L. ceylanicum*, Spring. The same.

14. *L. lucidulum*, Michx. No distinction of sterile and fertile zones: sometimes the fertile zone is very short; in some cases a single isolated sporangium is found in a sterile zone. The external differentiation of sterile and fertile zones is feeble.

16. *L. serratum*, Thunb. The foliage leaves of this species are unusually large. The size of the sporophyll varies: it may be fully the size of the foliage leaf, or it may be a mere tooth, subtending the large sporangium: but there is no obvious balance between the size of the sporangium and that of the sporophyll. This all shows that the larger size of the leaf does not necessarily depend on its being sterile; but usually the sporophylls are smaller than the foliage leaves, and this is specially the case at the upper limit of the fertile zones.

17. <i>L. firmum</i> , Mett.	} The condition in both is as in <i>L. Hamiltonii</i> : sporangia are found near the base of quite large plants.
18. <i>L. rigidum</i> , Gmel.	

Group of *L. taxifolium*.

20. *L. fontinaloides*, Spring. Alternating sterile and fertile zones without external differentiation, and of varying length.

21. *L. tetragonum*, H. & G. Usually sterile below, but after the transition, without change of form of shoot, the fertile form of shoot is maintained.

22. *L. myrsinites*, Lam. Alternating sterile and fertile zones: isolated sporangia found here and there in the sterile region.

25. *L. verticillatum*, Linn. fil. One of the least differentiated species, as regards sterile and fertile zones: there is no distinction between sterile leaves and sporophylls, while isolated sporangia are dotted here and there in the sterile region. In extreme cases there is no distinct fertile region at all, but only isolated sporangia among sterile leaves.

27. *L. setaceum*, Hamilt. }
28. *L. mollicomum*, Mart. } Alternating sterile and fertile zones.

30. *L. affine*, H. & G. }
31. *L. Lindeni*, Spring. } Alternating sterile and fertile zones.
32. *L. attenuatum*, Spring. }

35. *L. Trencilla*, Sodiro. This 'giant of recent Lycopodiaceous types' shows, in the Kew specimens, sporangia down to its very base: there is no differentiated strobilus.

36. *L. sarmentosum*, Spring. }
37. *L. linifolium*, L. } Alternating sterile and fertile zones,
usually with a sterile region at the
base: some isolated sporangia in the
sterile regions.

38. *L. dichotomum*, Jacq. There is usually a long sterile basal region: the sterile and fertile leaves are alike; the fertile zones are irregular: a few sterile leaves may intervene in a fertile zone, and isolated sporangia may be found in a sterile zone. At the limits abortive sporangia have been seen.

39. *L. taxifolium*, Swartz. There is a long sterile region at the base of the plant: above it alternating sterile and fertile zones: there is a tendency to fine off at the fertile apices, forming ill-defined terminal strobili, with smaller leaves: but the graduation is very slight and gentle. Mr. Baker notes for *L. passerinoides*, H. B. K., the leaves that 'subtend sporangia rather abbreviated.'

The above species constitute the sub-genus *Selago*, which includes mostly ground-growing plants: the characters are based on the sporangia being 'placed in the axils of unaltered leaves all down the stem¹.' The following are the most important points brought out in the above notes:—

1. Most species have a sterile basal region of considerable length: but sporangia have been observed down to the base of the mature plant, in *L. compactum*, and *Trencilla*, or close

¹ Baker, Fern-Allies, p. 8.

to the base in others, e.g. *L. firmum* and *rigidum*. There are, however, no records for young states of these species.

2. The sterile and fertile zones are imperfectly differentiated: this is illustrated by all the species.

3. The sterile and fertile leaves are mostly alike: and though the fertile leaves show in some cases a smaller average size than the sterile (*L. serratum*), this is not constantly the case even in that species.

4. Isolated sporangia have been frequently found in the otherwise sterile region, in the axils of leaves resembling the sterile leaves in size (*L. Selago*, *Hamiltonii*, *reflexum*, *miniatum*, *ceylanicum*, *lucidulum*, *myrsinites*, *verticillatum*, *setaceum*, *mollicomum*, *sarmentosum*, *linifolium*, *dichotomum*).

5. Sterile leaves have been found in the fertile zone (*L. dichotomum*).

6. Incompletely developed sporangia have been found at the limits of the fertile zones in *L. Selago*, and *dichotomum*: this is not readily observed in herbarium specimens, and most of the species have only been available in the dry state.

It is clear from these facts that the fertile and sterile regions are, in the sub-genus *Selago*, very imperfectly differentiated, a conclusion which has important bearings on any view as to the evolution of the genus.

Sub-genus II. **Sub-Selago.**

42. *L. carinatum*, Desv. The foliage leaves graduate by gentle steps to the sporophylls: the spike is terminal but not distinctly defined, and the lower limit of sporangia is not sharply marked. Straggling sporangia, and sporangia of smaller size, i.e. partially abortive, are found at the base of the spike. Occasional reversions from the strobilus to the sterile state have been seen in this species, a condition similar to that in the *Selago* group.

45. *L. gnidioides*, L. fil. The delimitation of the strobilus in this species is by no means exact: interruptions of the fertile zone occur at the base, and isolated sporangia are frequent, irregularly scattered in the sterile region below. The strobili often resume a vegetative condition at the apex.

In some Madagascan forms of this species there is a basal rosette of larger foliage leaves, from which springs at once the rather definite—branched strobilus condition, suggesting a comparison with *Phylloglossum*.

46. *L. dacrydioides*, Baker. The basal limit of the strobilus is ill-defined.

47. *L. squarrosum*, Forst. There is a specialization of the strobilus from below upward, as shown by the very gradual decrease in size of the sporophylls: at the base these equal the sterile leaves in size. The base of the fertile zone is not always sharply limited; occasionally isolated sporangia may be found below, in the sterile zone.

49. *L. Dalhousiaeaeum*, Spring. There is a considerable difference between foliage leaves and sporophylls, but the transition is gradual: sporangia appear first on the transitional leaves, about half-way between the two types. The strobili are apt to be interrupted by sterile zones, where however the leaves remain small.

The sub-genus *Sub-Selago*, introduced by Baker into Spring's system, shows the sterile leaves 'a little different from the bracts, but passing into them gradually. Sporangia aggregated into indistinct terminal spikes.' The few species thus grouped show many of the characters of *Selago*. All have a basal sterile region, but recurrence of sterile and fertile zones is rare, though occasionally seen (*L. Dalhousiaeaeum*): reversion from the fertile strobilus to a sterile state is more frequent (*L. carinatum*, *gnidioides*). The progressive diminution of size of the fertile leaves upwards is sometimes gradual (*L. squarrosum*); this was already indicated in *L. taxifolium*, in the *Selago* group: it is sometimes more sudden (*L. Dalhousiaeaeum*). Isolated sporangia in the sterile region are more rare, but do occur (*L. carinatum*, *gnidioides*, *squarrosum*): also partially abortive sporangia at the base of the strobilus (*L. carinatum*). Thus all the characters together show a very close similarity to what is seen in the *Selago* group, but with gradually increasing definition of the strobilus from the lower vegetative region.

Sub-genus III. *Lepidotis*.Group of *L. inundatum*.

50. *L. inundatum*, L. The transition from sterile to fertile leaves is rather sudden, the fertile leaves being slightly smaller, and widened at the base. The basal region of the strobilus shows sporangia abortive in various degrees, some consisting merely of a mass of vegetative cells, containing no spore-mother-cells: these abortive sporangia occupy the normal position, and intermediate steps lead on to the normally developed sporangia above. There is usually no irregularity of the spike in this species: in certain varieties it is very lax in habit, and the spike is carried up on a long stalk (var. *pinnatum*, Chapm., and *Bigelovii*, Tuckerm.), a habit suggestive of *L. alopecuroides*.

51. *L. Drummondii*, Spring. Specimens in Kew from Baron v. Müller show much elongated and interrupted spikes, with alternating sterile and fertile zones.

52. *L. alopecuroides*, L. The transition from the vegetative to the fertile region is not abrupt as regards leaf-form: no interruptions observed. In habit like a large *L. inundatum*.

In the *inundatum* group, which inhabits swampy ground, the differentiation of the strobilus in form is still only slight, while abortive sporangia are found at the base (*L. inundatum*): *L. Drummondii* even approaches *Selago* in its alternation of successive sterile and fertile zones.

Group of *L. Phlegmaria*.

55. *L. nummularifolium*, Blume. Fertile spikes may pass again into foliage shoots, and these again to fertile spikes: this seems not uncommon.

56. *L. aqualupianum*, Spring. Ditto.

60. *L. varium*, R. Br. The strobili of this species are apparently well defined, and it is therefore placed in the group of *L. Phlegmaria*; but Sir J. Hooker's specimen from the Auckland Islands, in the Glasgow herbarium, shows that sporangia of normal size are present far below the apparent limits of the strobilus. The latter are 'sub-tetragonous,' with broad ovate-acute sporophylls: but the sporangia

are found also in the axils of large 'foliage leaves' below. This was verified also in other specimens.

61. *L. subulatum*, Desv. The condition here is the converse of *L. varium*. The strobili are well defined, their leaves being broad and ovate, while the foliage leaves are lanceolate-acuminate: but the lower part of the strobilus with leaves of the sporophyll-type may have no sporangia, and higher up irregular zones may also be sterile: the apex of the strobilus may revert to the vegetative type of leaf.

62. *L. ophioglossoides*, Lam. (incl. *L. fertile*, Baker), shows transitions of the fertile strobilus to the sterile state.

63. *L. pinifolium*, Blume, ditto.

67. *L. Phlegmaria*, L. There is a sharp contrast between the large foliage leaves and the small sporophylls, and the transition is usually sudden, though intermediate leaf-types do occur. Usually the strobili are continuously fertile, but at the branchings the first leaves above may be sterile, though of the small sporophyll-type. Transitions from the fertile to the sterile type of shoot, and back to the fertile, are not uncommon.

It is seen from the above notes that though in the *Phlegmaria-group*, which are epiphytic, the spikes are 'slender, dichotomously forked, with bracts very different from the leaves¹, isolated sporangia may be found in the vegetative region (*L. varium*), and there may occasionally be alternating sterile and fertile zones (*L. nummularifolium*), while leaves of the sporophyll type may develop no sporangia (*L. subulatum*, *Phlegmaria*). Transitions from the fertile strobilus to the larger leaved foliage shoot are frequent (*L. nummularifolium*, *subulatum*, *ophioglossoides*, *pinifolium*, *Phlegmaria*). Thus the differentiation of the strobilus is one of external form, rather than a rigid difference of intimate character. The converse conditions of *L. varium* and *L. subulatum* show that the difference in size of sporophylls and foliage leaves is not due directly to correlation in the individual: other cases might be quoted supporting the same conclusion, notably *L. serratum*.

¹ Baker, loc. cit., p. 8.

Group of *L. cernuum*.

In the *cernuum* group of ground-growing species the strobili are clearly defined. Transitions from the strobilus back to the vegetative shoot are decidedly rare: none were observed in *L. cernuum* itself, in a very large number of specimens: a slight indication of it was seen in *L. obscurum*, but none in *L. casuarinioides*.

Group of *L. clavatum*.

These are all terrestrial, with trailing habit, and ascending branches, which bear well-defined strobili: reversions from strobilus to vegetative shoot have not been observed. A comparison of the species illustrates the way in which the strobilus may have come to be lifted on a long pedicel above the ground, with the result of a better dissemination of the spores: this is shown by the following notes.

76. *L. Sprucei*, Baker. This species having solitary spikes sessile on leafy branches, connects the group with *L. inundatum*.

77. *L. magellanicum*, Sw., has also spikes 'sessile or nearly so,' and 'often many to the branch,' as in *L. clavatum*.

80. *L. annotinum*, L. The leaves at the base of the strobilus are frequently smaller than either foliage leaves or sporophylls, but they occupy no specially elongated zone: the leafy branches, which rise from the creeping rhizome and bear the strobili, attain a height of six inches.

78. *L. contiguum*, Klotzsch. The zone may be a quarter of an inch in length: the strobilus simple or sometimes branched.

81. *L. diaphanum*, Sw., has a considerable zone of narrower linear leaves between the strobilus and the larger foliage leaves. The strobilus is simple or branched.

84. *L. clavatum*, L., has an elongated stalk, of length varying from two to six inches, covered with distant appressed scales, i. e. the axis has lengthened, and the scales are correlatively reduced. *L. aristatum*, H. B. K., is a form with specially elongated stalk, and several spikes.

86. *L. paniculatum*, Desv. The stalk, nearly a foot long, may arise direct from the rhizome, and bears many branched spikes.

In this series of ground-growing forms, the intercalation of a peduncle, with small distant scales, between the larger-leaved foliage shoot and the definite strobilus, is indicated. The question whether the peduncle is directly derived from the basal part of the strobilus, or from a specialized part of the already sterile foliage-region, may be left open: but as sporangia are not found on it, nor even any vestiges of arrested sporangia, the latter seems the more probable source of the peduncle. The importance of it in ground-growing forms is obvious.

Group of *L. laterale*.

The strobilus is well differentiated from the sterile shoot.

88. *L. diffusum*, R. Br., 'intermediate between *L. laterale* and *L. magellanicum*'. After clear cases of dichotomy, the one branch continues sterile growth, the other is fertile.

89. *L. laterale*, R. Br., the same behaviour of the branches is seen, but more pronounced: sometimes both branches are sterile: in some specimens the successive branches on the same side are fertile, giving a peculiar sympodial effect.

Sub-genus IV. **Diphasium**.

The strobilus is well differentiated from the sterile shoot. The peculiarity is in the bilateral development of the latter.

90. *L. carolinianum*, L. In some forms differs only in minor degree from *L. clavatum* or *L. inundatum*, as regards leafage; but some forms, e. g. *L. sarcocaulon*, Welw., show extreme cases of dorsiventrality.

92. *L. complanatum*, L., often regarded as merely a dorsiventral form of *L. alpinum*.

93. *L. scariosum*, Forst., sometimes shows the strobilus fining off to an attenuated, vegetative apex, but no complete return to the foliage type of shoot.

94. *L. volubile*, Forst. A highly specialized, scandent, dorsiventral shoot, with definite strobili, which show no return to the vegetative type.

¹ Baker, loc. cit., p. 27.

GENERAL THEORETICAL POSITION BASED ON COMPARISON
OF THE GENUS LYCOPODIUM, AS REGARDS DISTRIBUTION OF SPORANGIA.

The theoretical bearing of such facts as the recurrence of sterile and fertile zones in certain Lycopods, and the existence of abortive sporangia at the limits of the fertile zones, was pointed out in the first part of my studies¹, and again in a later paper in the *Annals*². The facts being now amplified by examination of a larger number of species, and of specimens, the conclusions which may be drawn will be stated in an extended form.

First it will be well to re-examine the theoretical position as regards abortive parts generally. It is possible to look upon any imperfectly developed part as either in the up-grade of development—as something *nascent*; or in the down-grade of development—as something *evanescent*; and these ideas themselves may be applied in either of two senses, according as they are taken to refer to the individual or to the race. There are thus four possible views which may be taken of any imperfectly developed part:—

- (1) that it is *nascent* in the *ontogeny*;
- (2) that it is *evanescent* in the *ontogeny*;
- (3) that it is *nascent* in the *phylogeny*;
- (4) that it is *evanescent* in the *phylogeny*.

These distinctions may not be admitted by those who assume that the ontogeny is a direct and complete index of the phylogeny, that the parts first formed in the individual were also the first to appear in the evolution of the race. This 'recapitulation theory' may be consistently entertained for the sporophyte by those who hold that the alternating generations of the Archegoniatae originated by differentiation of homologous phases³: to them the view is possible, though I am not aware of its having been definitely expressed, that spore-production may have been in the evolution a sort of

¹ Phil. Trans., 1894, B., p. 535.

² Annals of Botany, vol. viii, p. 343.

³ See Scott, Presidential address to Sec. K, Brit. Assn. Report, 1896, p. 996; where the necessary literature is quoted.

afterthought, a mode of propagation taken up by already existent vegetative parts. There is, however, a comparative obstacle to this view: for spore-production is a characteristic of the simplest sporophytes: and if the plants of the past in any degree resembled the plants of the present, spore-production must have been, even on an homologous theory, an early event in evolutionary history. But to those who hold the antithetic theory of alternation, that the sporophyte is the result of amplification of the zygote, and that spore-production was its first end, and was, just as it is now seen to be, a constantly recurring feature throughout descent¹, a recapitulation theory is plainly inconsistent with their view². For *ex hypothesi*, in the simplest cases the spore-production preceded the vegetative development of the sporophyte, though in development of the individual, in the more advanced cases, the vegetative system precedes the spore-production.

The question of priority in the history of descent of sporophylls and vegetative leaves cannot then be settled summarily by the statement that the latter appear first in the ontogeny³: neither can it be decided by any detailed comparison of the two leaf-forms as regards individual development⁴: an hypothesis that the foliage leaf is a sterilized sporophyll is based just as much upon the fact of the similarity of development of the two leaf-forms, as the converse view that the sporophyll is an altered foliage leaf. Nor will the record of an infinity of intermediate forms, half sterile and half fertile⁵, nor the proof that experimentally the sporophyll can be converted into a foliage leaf⁶, carry us any further than to show the intimate relations of the two. These facts do not touch the question of phylogenetic priority.

¹ See Bower, Presidential address to Sec. K, Brit. Assn. Report, 1898, p. 1031.

² This was fully pointed out by me in the *Annals*, vol. vi, p. 372.

³ Goebel, *Science Progress*, 1895, p. 120.

⁴ Glück, *Flora*, 1895, Heft 2, p. 383.

⁵ Glück, *loc. cit.*, p. 384.

⁶ Goebel, *Annals of Botany*, vol. vi, p. 359; also *Ber. d. Deutsch. Bot. Ges.*, 1887. Atkinson (*Bot. Gaz.*, vol. xxii, p. 220) made similar experiments, and from similar results finds the converse view to be still tenable.

But a valuable clue for decision of the case for the leafy sporophyte is derived from the analogy of the simpler Bryophytes. The sporogonium of *Riccia*, with no seta, is currently accepted as the most primitive type¹. A comparison of successively more complex types indicates the intercalation of a vegetative phase, the seta, before the spores are formed. The same is the case in other Bryophyta, and even in Mosses where the seta often produces a considerable vegetative development for nutritive ends. Are we to assume in these cases that the vegetative seta, because it precedes the capsule in origin in the individual, preceded it also in the phylogeny? We can only conclude from their comparative study that spore-production, which is the constant feature in them all, has been deferred, by a later intercalated phase. If that be so, then the order of appearance of the parts of the individual sporogonium is not an index of the order of their appearance in the history of the race.

The same argument will hold for the whole plant of a *Lycopodium*, and an interesting analogy is to be traced between the successive vegetative and fertile phases of a Bryophyte sporogonium, and of a simple Lycopod. In such a Moss as *Funaria* or *Polytrichum*, the archesporium appears in longitudinal sections of young sporogonia, as a definite row of cells on either side of the columella. It is impossible at first to tell in those rows of cells the exact limit where spore-development will actually begin. Below that point the cells of the row develop sterile, above it fertile; but in either case the segmentations which lead to them are the same. Passing to the apex, the archesporial row is continued beyond the extreme limit of fertility; structurally the possibility of further spore-production seems to be there, but arrested. In the Lycopod a similar succession of phases is seen, but complicated by the fact that appendicular organs are borne: the lower sterile region may be compared as regards its physiological condition with the seta, though here more

¹ Goebel, *Organographie*, ii, pp. 321, 328.

definitely nutritive in function¹: the strobilus with the sporogonium: the abortive sporangia at the base with the arrested archesporial region at the base of the capsule: the abortive sporangia so commonly found at the apex of the strobilus with the arrested archesporial region, which passes upwards into the operculum. There is first in each case a vegetative phase, which is nutritive; this merges into a fertile phase, which again is arrested above, being probably in each case limited by nutritive supply. On the antithetic theory, in either case the spore-production was the prior function in the race; in either case that function was delayed in the individual by a later intercalated vegetative phase. The prevailing view which regards the evolution of the sporophyte in the two great series of the Archegoniatae as following from the very first a distinct course of development is probably correct, in the sense that specialized Bryophyte sporogonia did not give rise to Pteridophyta; but the analogies as regards balance of the vegetative and spore-bearing phases should not be lost sight of, on the superficial ground that appendicular organs are present in the one and not in the other. For both series have equally been subject to the fundamental laws of nutrition, which have dictated these successive phases.

Our first duty will then be to interpret those parts which are found about the transition from the vegetative to the fertile region in *Lycopodium*, and see what bearing they may have on our views of the origin of the vegetative region in that genus. But in doing so we shall start with the clear understanding that though certain parts are formed first in the

¹ It seems hardly necessary to state that no exact morphological homology is here implied, but merely a comparison of the successive phases of nutrition and spore-formation. The specialized sporogonium of a Moss is not looked upon as the progenitor of any race of vascular plants. As I have been credited in various quarters, and on insufficient grounds, sometimes quite misunderstood, with the opinion that a Moss sporogonium was the source from which a leafy sporophyte originated, I wish to expressly disown such an opinion. A parallelism in method of morphological advance may be traced, without any recognition, in such series as show it, of any true affinity.

individual, they are not on that account to be accepted as of prior existence in the history of the race.

Of our four possible views which may be taken of imperfectly developed parts, we may now consider which is applicable to those imperfect sporangia which have been found at the base of a strobilus or fertile zone in various species of *Lycopodium*. In a sense they may be looked upon as nascent in the ontogeny, in so far as, starting from leaves which show no sign of sporangia at their base, we pass upwards to similar leaves presenting a sporangial rudiment, and on through successive phases of increasing size and sparing spore-production to the fully formed sporangium. Some deficiency of nutriment has probably determined their incomplete development in the lower region, but in the first stages of even the smallest rudiment the potentiality of complete development probably was once there, though not realized. Thus in a sense they may be styled nascent as regards the individual development. But are they to be looked upon as nascent in the phylogenetic sense? Does the progression from a minute vegetative papilla, through successive meagre stages of spore-production, to the fully formed sporangium, in any sense indicate the stages of actual evolution of the Lycopod sporangium? This question must be answered in the negative. In the first place, the analogy with rudimentary stamens and pistils of Angiospermic flowers is obvious: in either case the rudimentary part is a mere parenchymatous papilla, which however occupies the correct position of the part which it represents. We must regard these imperfect sporangia as reduced rudiments, just as much as the abortive stamens of the Scrophulariaceae, or the abortive pistil of *Lychnis dioica*. And secondly, the whole weight of evolutionary probability is against the view that the progression in question is an upward progression: for these imperfect sporangia are present on fully developed sporophylls: in their simplest forms they bear 'the plain stamp of inutility'¹: like other rudimentary parts, they are highly variable². The complete absence of

¹ Origin of Species, chap. xiv, p. 397.

² Ibid., p. 119.

them in the vast majority of the leaves below the fertile zone may be explained on the general principle of economy, by which parts no longer functional are liable to obliteration¹. On such grounds as these the opinion may be surely based *that the imperfect sporangia at the base of the strobilus are vestigial organs, and not indicative of an upward evolution, leading in the race to the complete sporangium.*

Goebel, in his *Organographie*², has pointed out that 'we must, however, guard against considering all arrested organs as being descended from organs which were developed in the ancestors of existing forms.' He alludes to certain inflorescences, and remarks that it is 'quite a general rule that many more primordia of organs are formed than become functional.' In this matter I think that *Lycopodium* in its simple way is instructive. We find incompletely developed sporangia, both at the apex and the base of the strobilus (e.g. of *L. inundatum*), these, however similar in appearance, seem to have had a different phylogenetic history. For reasons already stated the basal sporangia may be held to be arrested, and vestigial as regards descent, and that in the ancestry they were represented by fully formed sporangia. But the series of successively smaller sporangia at the apex may be regarded as primordia of organs which may have never become functional in the ancestry: they are subtended by leaves of an arrested type, imperfect like the sporangia which they bear. And this is, indeed, the criterion by which such cases may be judged. Many apical buds, like those of *Lycopodium*, have an apparently unlimited power of forming primordia; but fail to mature them all: these rudiments might be described as phylogenetically nascent, or supernumerary; while sporangia or buds at the base of the fertile region would be properly regarded as phylogenetically evanescent,—as representing parts which in the history of the race had been accustomed to come to functional maturity. Thus in the case of *Lycopodium* we acquire the idea of *a zone of reproductive activity, limited below by phylogenetically evanescent or*

¹ Origin of Species, chap. iv, p. 117.

² Organographie, Engl. ed., p. 60.

vestigial parts, above by phylogenetically nascent or supernumerary parts. Again, the comparison may be made with a Moss-sporogonium, and there also a fertile zone is found, with a vegetative region below: the series of cells of the archesporium is continued beyond the region of actual fertility both above and below: and the cells at the lower limit may similarly represent a phylogenetically evanescent archesporium, those at the upper limit a phylogenetically nascent region. As regards the succession of vegetative and reproductive phases, and their probable evolutionary origin in the sporophyte generation, the cases are alike¹. What it is in such cases that determines where the limits of the fertile zone shall be is obscure; but the cause is doubtless related closely to nutrition¹.

By comparison of living species of *Lycopodium* we see that the fertile zone is not always located at the same level of the plant as a whole: it is sometimes preceded by a shorter, sometimes by a longer vegetative region. An idea can thus be arrived at of *the shifting of the fertile or spore-producing zone*. The biological significance of this shifting is obvious, for any advance of the zone to a higher point by abortion of sporangia, while the sporophylls remain in a vegetative capacity only, provides a larger vegetative zone below for purposes of nutrition.

In most species of the undifferentiated *Selago* group, as in all the more specialized species of *Lycopodium*, there is a considerable basal region which is sterile. This region is however variable in length; indeed in some species of the *Selago*-group, notably in *L. compactum* and *Trencilla*, sporangia are found quite down to the base of the mature plants; here then the whole of the mature plant is virtually a strobilus. Comparison of these extreme cases with those which show alternating sterile and fertile zones leads fairly to the conclusion,

¹ Parallel examples might be quoted from the cones of Gymnosperms, and the inflorescences of many Angiosperms; but I prefer for the present to discuss only those simpler cases where the questions in hand are not complicated by high specialization.

that the whole leafy plant in the mature state is potentially, as it is in these actually, fertile. Attention is then thrown back to embryonic plants, and the question arises, how early may the sporangia make their first appearance on the young sporophyte?

We have as yet no information as to the embryology of any of those extreme forms where sporangia are borne down to the base of the mature plant; and indeed of the whole *Selago* group, with its comparatively undifferentiated sporophyte, it is only in one species that the embryonic details have been observed, viz. in *L. Selago*. Bruchmann¹ has given an account, with drawings, which supplies the facts. The prothalli of this species may be either buried in the soil, or superficial, in which latter case they may be deep green. But Bruchmann² remarks that 'it appears to be of great advantage to the seedlings, which are not adapted for an underground growth, to arise as near to the surface of the soil as possible, so that their first leaves may easily reach the daylight.' This species then is not one of those specialized cases like *L. clavatum*³, and *L. annotinum*, with deeply buried prothallus, but more nearly shares with *L. cernuum* and *L. inundatum* what was probably the primitive state, viz. with a superficial, chlorophyll-containing prothallus. Bruchmann⁴ figured a large number of varying cases of the prothalli of this species, at different levels: some at the surface of the soil, others below, with their seedlings attached; and from these, together with his text, certain important facts emerge. The first period of development of the embryo, that is the initiation of the fundamental structure of the germ in *L. Selago*, corresponds to that in other species of the genus⁵: but in

¹ Ueber die Prothallien und die Keimpflanzen, &c., Gotha, 1898, p. 97, Pl. VI, VII.

² Loc. cit., p. 85.

³ For an additional description of the facts for *L. clavatum*, together with an excellent comparative discussion of the prothallus and embryo in other species, from the biological point of view, see W. H. Lang, Annals of Botany, vol. xiii, p. 279.

⁴ Loc. cit., Pl. VI, Figs. 1-26.

⁵ Bruchmann, loc. cit., p. 98.

the further development important differences from other European species arise, and the embryo in its later form resembles that of *L. Phlegmaria*. The foot remains small as a conical continuation of the large suspensor: there is no protocorm, nor are there any protophylls of the *Phylloglossum* type: the first leaves are green, and resemble the foliage leaves of the mature plant of *L. Selago*: these are carried up to the surface of the soil, whatever the position of the prothallus—buried or at the surface—by a proportional intercalary growth in the hypocotyl, which is directly continuous with the foot¹: the first root is formed near its base. The first branching of the axis occurs in plants which have about six or seven developed leaves: 'after the second branching these seedlings may produce spores, while in other native species only quite old plants proceed to spore-development².' These characters, together with those of the sporophyte, are recognized by Bruchmann as showing that *L. Selago* is not near to any of the species native in Germany³.

Perhaps the most important of these facts is the early appearance of the sporangia on the seedling of *L. Selago*. It shows, in the only species of the *Selago* group yet examined embryologically, that early spore-production goes along with the undifferentiated condition of the whole plant. The stele also of the mature plant is of simpler structure, and probably more primitive type than those of other species whose embryology and anatomy are both known. These characters together indicate the primitive nature of the group, and suggest the further question, is the embryology also of *L. Selago* to be regarded as a primitive type, compared with that of other Lycopods, and if so what will the effect be on our views regarding *Phylloglossum* and the theory of the protocorm?

Before Treub's paper in which this term was introduced, and the theory of the protocorm enunciated⁴, the 'embryonic

¹ Bruchmann, loc. cit., Pl. VI, Figs. 1-26, and Pl. VII, Figs. 41-43.

² Ib., p. 100, and Pl. VI, Figs. 26-27.

³ Loc. cit., p. 102.

⁴ Buitenzorg, Annals, vol. viii, p. 1.

tubercle' recognized in *L. cernuum* was regarded as a foot, which had escaped from the prothallus: it is still spoken of by Bruchmann¹ in that way, and the type of embryo of *L. inundatum* is described as 'freifüssig,' in which the embryonic foot is developed outside the prothallus. But Treub showed that the embryonic tubercle is not of the same origin as the intra-prothallial foot: the latter originates from the first or lower tier of the embryo, the tubercle from the upper, which gives rise also to the cotyledon and stem. The embryonic tubercle, thus shown by origin not to be the true foot, was regarded by Treub as an organ which played an important part in the passage of the sporophyte to a state of physiological independence. He regards it as a primitive, not a reduced structure, and introduced the term 'protocorm,' recognizing in it a preliminary stage of development of the young Lycopod plant. Those cases in which no obvious protocorm is developed are explained on the ground of its disappearance, and because of their epiphytic saprophytism (*L. Phlegmaria*, *carinatum*, *Hippuris*, *nummularifolium*) they are held to be more recent types than those which show a protocorm. Naturally in *Phylloglossum* the tuber itself is regarded as a protocorm, repeated over and over again, and on Treub's theory of the protocorm it would thus take a place as an embryonic type still playing a considerable rôle.

But now the question is, How will the embryology of *L. Selago* fall in with this view? It is a ground-growing species, in which neither the prothallus nor the sporophyte are highly specialized. But the protocorm is absent, there being no swelling at the base of the hypocotyl until the appearance of the first root. These facts seem to me to necessitate a reconsideration of the protocorm and protophylls: are they really primitive structures, general in the ancestry of all Lycopods, or the result of a vegetative adaptation, which has made its appearance in certain species only; formed early in the individual plant, but relatively late in the phylogeny; and not a general character for the whole race?

¹ Bruchmann, loc. cit., p. 102.

I think the latter view is the more probable one. It is apparent from the various memoirs on embryonic Lycopods that the initial plan of the embryo is essentially the same for all the species described; and that the differences depend upon different proportional development of the parts, and difference in the time of their first appearance. Where one part is largely developed at an early period, others are apt to be delayed, or to be developed on a smaller scale: in fact the principle of correlation holds in the embryo as elsewhere. As examples, in *Phylloglossum* (which may with propriety rank with the embryonic Lycopods) the large protocorm is formed before either protophylls, roots, or strobilus are initiated. In *L. cernuum* and *inundatum* the root and leafy shoot appear late, though the protocorm and protophylls which appear early are of relatively large size. In *L. clavatum* and *annotinum* the foot is very large and the axis early developed, but the first leaves are small and the first root appears rather late. On the other hand, in *L. Selago*, where no disproportionately large protocorm or foot is formed, axis and leaf are defined relatively early, and the root soon follows. Are then the protocorm or the specially enlarged foot really primitive parts of the embryo, or disturbing influences introduced only in special cases to meet special needs? Was it once universal in Lycopod embryos, and is it therefore necessary to explain by abortion the absence of such parenchymatous swellings in certain species¹, and especially in *L. Selago*, which, as we have seen, shows in other respects

¹ Dr. Treub has quoted certain embryos of *L. Phlegmaria*, as showing what he regards as a rudimentary protocorm (Buit. Ann., viii, p. 32). In his paper on this species (Buit. Ann., v) he has shown on Pl. XXVI, in a series of embryos, a rounded swelling (*R*), in the position in which the protocorm appears in the *cernuum* type: it may, under circumstances, form rhizoids (Fig. 4), but soon the true root forms at the apex of the protuberance. It is possible that this may be a rudimentary protocorm, but I do not think the facts conclusive; for the root appears exogenously just at the point of greatest convexity of the swelling; moreover, in *L. cernuum*, as in *Phylloglossum*, which have typical protocorms, the root does not appear on the tip of the protocorm (which would thus be its position if the swelling in *L. Phlegmaria* be really a protocorm), but in the upper region nearer the leaves.

such primitive characters of the sporophyte? Taking into account the characters of its mature sporophyte both external and internal, I think we must regard *L. Selago* as primitive, and that it is so in the embryo as well as in the more mature sporophyte: its embryo quickly forms assimilating leaves, and its early nourishment is thus simply provided for, without the formation of any protocorm or protophylls.

The following considerations may help towards some alternative view of the protocorm and protophylls more in accordance with the fact of their absence in *L. Selago*, which on the above grounds we regard as a primitive species. The embryo Lycopod seems prone to parenchymatous swelling; two such swellings, somewhat similar in structure, but differing in place of origin and in function, are known, viz. the enlarged 'foot' of *L. clavatum* and *annotinum*, which originates from the lower tier of the embryo, and is intra-prothallial; and the protocorm of the *cernuum* type, which originates from the upper tier of the embryo, and is extra-prothallial. The former acts as an haustorium, the latter as a place of storage. A genus which shows two types of parenchymatous swelling in two distinct types of embryo, while both are absent from other species, cannot be expected to have ever had one of those as a constant feature in its ancestry. This consideration makes me doubt any general application of the theory of the protocorm in the genus *Lycopodium*. I should look upon these parenchymatous swellings, whether of the enlarged intra-prothallial foot or of the protocorm, as opportunist growths rather than as persistent relics constant in the ancestry. *Phylloglossum*, with its large protocorm, would then be the extreme type of a line of embryological specialization, not a form preserving the primitive embryological characters of the whole race¹.

¹ This discussion leads me back to a similar one written in 1882, in a paper on *Gnetum Gnemon* (Q. J. Micr. Sci., xxii, p. 277), in which the conclusion was arrived at that the foot in vascular plants at large is not to be regarded as a clearly defined morphological member; it may rather be looked upon as a swelling of tissue, which arises only when and where required for the nutrition of the embryo. Doubtless in certain circles of affinity there is a degree of constancy in position and

In seeking to understand the nature of the protophylls it is a material fact that they are not sharply marked off from the typical foliage leaves: in embryos of the *cernuum* type they merge gradually into the normal leaves, which in their turn pass on into sporophylls. And even in *Phylloglossum*, where usually the distinction is more marked, intermediate steps have been seen between the protophyll and the sporophyll. Another fact of importance is that protophylls are absent from some Lycopod embryos, and when present are only found on the protocorm. The relation of these parts, the protophyll and the protocorm, are evidently close; and as the protocorm may, on my view, be regarded as a special turgid development of the lower part of the stock, so the protophylls may be regarded as turgid types of leaf, the transitional forms to the normal foliage giving some idea of the steps of their specialization.

In the Lycopodineae we have thus three categories of leaf which merge into one another. We have seen that in the *Selago* type the sporophylls do not differ from the foliage leaves; that the first leaves of the *Selago* plantlet are normal foliage leaves; and that sporangia may be produced after the second branching of the young plant. It does not seem a strained interpretation of these facts that in *Selago* all the leaves from the first are potential sporophylls; but that those first formed are usually sterile by complete abortion of sporangia. We may then conceive the primitive embryo of *Lycopodium* as of the *Selago* type; with a hypocotyledonary stem which forms the first root near its base; with no protocorm nor protophylls, nor greatly enlarged foot; but connected by a suspensor and small foot with the prothallus, and its

origin of such haustorial structures, but the inconstancy in vascular plants at large prevents too precise generalization. Dr. Treub, referring to this question (Buit. Ann., v, p. 130), remarks that my conclusion goes too far, in denying the morphological value of the foot in Vascular Cryptogams. But the further examples since described confirm me in the opinion. Goebel (*Organographie*, ii, p. 450) suggests that the term foot should be dropped, and haustorium substituted: this is a colourless term, which does not suggest any morphological identity for the parts included under it, but only physiological correspondence. On this ground the suggestion may be upheld.

first leaves early exposed as assimilating leaves; these, however, do not differ in form from the early sporophylls with which they are probably homogeneous, though sterile by abortion of their sporangia. Starting from this prototype, the following modifications of the sporophyte are indicated by comparison of living species of *Lycopodium*. In those types with a protocorm (which are all more specialized sporophytes than *Selago*, having their sporangia formed later in the individual, and usually more strictly localized in strobili), the lowermost leaves, already sterile, have been developed as protophylls, which though appearing first in these individuals, would represent a relatively recent modification of leaf structure¹. The fertile region, which is continuous in some species, is in most of the *Selago* group interrupted by irregular sterile zones, an arrangement which provides for more adequate nutrition, for which frequently a long sterile basal region precedes the fertile (sp. 6, 7, 38, 39, &c.). The sterile and fertile zones usually differ only in the absence or presence of sporangia, but there is, in some species of the *Selago* group (sp. 9, 17, 39), evidence of a fining off of the sporophylls to smaller size than the sterile leaves, with the result of a partial definition of a fertile strobilus: this becomes more apparent in the *sub-Selago* group (sp. 42, 47, 49), but it is in the *Lepidotis* group that the strobilus becomes a definite terminal cone (sp. 50, 52), though still liable to be interrupted (sp. 51). In the *Phlegmaria* group the strobilus is marked off more sharply by the sporophylls being small; but still occasional sporangia may be found outside the strobilus (sp. 60), while leaves of the strobilus may be barren (sp. 61, 67); the strobilus may also be continued into a shoot of the foliage type; clearly the distinction of vegetative and fertile regions is not yet defined absolutely. But in the *cernuum* group the definition is more exact. In the *clavatum* group of ground-growing forms again the strobilus is well

¹ The terms 'protocorm' and 'protophyll' may well be retained, but with the understanding that their priority is in the development of the parts of the individual, not of the race.

defined, and certain members of the group illustrate a further specialization in accordance with their ground-habit, the strobilus being carried up on an elongated peduncle with small scale-leaves, an obviously useful device to secure a wider distribution of the spores. Another line of specialization results in the dorsiventral vegetative shoot, as in *Selaginella*, and this culminates in the climbing species, *L. volubile*, with its large and branched foliage system and clearly defined strobili. The whole genus shows in its living species the lines of specialization fairly indicated by gradual specific steps, starting from an undifferentiated strobilus, and attaining first a clear differentiation of the strobilus from the vegetative region: the latter may become in a high degree adapted to its environment; the former probably retains more truly the primitive condition of the whole shoot. Thus a comparison of the living species indicates that there has been a shifting onwards of the spore-producing zone and progressive intercalation of a vegetative zone, comparable to that indicated in the Mosses. In both series there is a strong physiological probability that such a differentiation should take place, as a nutritive advantage is gained, and in some cases the better provision for dispersal of the spores is secured; both advantages being the result of comparatively slight morphological changes.

The facts relating to partially or completely abortive sporangia in the genus *Lycopodium* are fairly intelligible, owing partly to the undifferentiated state of some of the species of the *Selago* group, partly to the considerable number of species in the genus. It is not to be expected that the matter will be as obvious in other Lycopodineae; still, abortive sporangia are found in other genera, which are susceptible of similar interpretation. Such examples as have been found will now be noted.

SELAGINELLA.

In this genus the strobili are definitely marked off from the vegetative region; no case of alternating sterile and fertile zones is recorded, but in some species the fertile spikes revert

at the apex to a vegetative character. Abortive sporangia have been seen at the base of the strobilus in *S. spinosa*, P. B.¹, and in *S. Martensii*, and would doubtless be found in many species², but no isolated sporangia have been seen in the sterile region.

The arrested sporangia at the base of the strobilus will bear the same interpretation as those in *Lycopodium*. Clearly the genus is in this, as in other respects, more specialized than *Lycopodium*.

ISOETES.

Wilson Smith³ has pointed out in the case of *I. echinospora* that the sterile leaves differ from the sporophylls in their smaller size. But a closer study of the sterile leaves almost always 'reveals the presence of aborted sporangia.' This observation led me to look over my old sections of *Isoetes lacustris*, with the result that sporangia in various degrees of abortion were found: in proportion as the spores are few in these, the sterile tissue is relatively bulky, but many of the sporangia remain quite small. Dissections showed that in the majority of leaves that are apparently sterile a rudimentary sporangium is really there, in the normal position.

Wilson Smith remarks further (l. c., 324) that 'the occurrence of aborted sporangia on so many of the sterile leaves shows that all the leaves are potentially sporophylls, and suggests the probability that *Isoetes* has retained a more primitive form than any other vascular plant.' This is clearly going too far⁴; but none the less the fact that most of the leaves show abortive sporangia is interesting in relation to the question in hand. The irregular recurrence of the sterile and fertile zones is similar to that seen in the *Selago* group of *Lycopodium*. It would be important to know how early

¹ Glück, Sporophyllmetamorphose, p. 355.

² See Studies, i, Phil. Trans., 1894, p. 522. I found them in all of the few species adequately examined.

³ Bot. Gaz., 1900, vol. xxix, p. 323.

⁴ Compare the remarks of Scott and Hill, Annals of Botany, vol. xiv, p. 443, &c.

in the embryo the first traces of a sporangium may appear. They are certainly absent from the first-formed leaves of the young plant¹.

PSILOTACEAE.

In *Tmesipteris* there is commonly a sterile region of some length at the base of the aerial shoot; in its upper part, fertile and sterile zones may alternate without any definite regularity. The zones are, however, excessively irregular in *Tmesipteris*; thus an odd synangium, or two or three, may be interpolated in a sterile region, or odd sterile leaves may occur in a region that is mostly fertile, a condition which has been found in some species of *Lycopodium*.

The length of the leaf does not depend upon the presence or absence of a synangium; fertile leaves often equal in length the sterile ones. But there is usually a general diminution of the size of the appendicular organs at the upper limit of the fertile region (especially where that region is long) which affects both organs alike. This condition resembles that of the less differentiated species of *Lycopodium*, of the *Selago*, and *sub-Selago* groups.

Arrested or imperfectly developed synangia are not uncommon, especially at the limits of the fertile zones². They appear as small growths in the normal position on otherwise fully developed parts. They are susceptible of the same interpretation as the imperfect sporangia of *Lycopodium*.

In *Psilotum* the distribution of sterile and fertile regions resembles that in *Tmesipteris*, successive zones being found on the same branch, while the various branches of one shoot show a synchronism, in the limits being at the same levels in each; as is the case also in some of the *Selago* group of *Lycopodium*. Partially or completely abortive synangia are found especially about the limits of the fertile zones; and Solms-Laubach³ notes the intermediate types of leaf between

¹ Campbell, Mosses and Ferns, Figs. 149-153.

² Bertrand, Arch. Bot. d. Nord, vol. i, p. 475; Bower, Phil. Trans., 1894, B., p. 544, and Pl. LII, Figs. 149-153.

³ Solms-Laubach, Buit. Ann., vol. iv, p. 174.

the simple and the double-bladed forms ; he remarks, however, that where they are more or less deeply cut or completely bifurcate there is almost always the rudiment of the synangium, initiated but not brought to full development, in the form of an outgrowth usually of brownish colour.

These facts for the Psilotaceae are plainly comparable with those in *Lycopodium* ; and similar arguments might be based on them. But the case here cannot be so well appreciated, nor such clear conclusions drawn, owing to the fact that the group is isolated and represented by few living species ; and secondly, that the embryology is not known, while in the well-grown plants there is a long basal vegetative region before synangia first appear.

It is thus seen that abortive sporangia are found in all the genera of Lycopodineae, and, with the exception of *Phylloglossum*, at both the upper and lower limits of the fertile zone ; often also within the fertile zone itself. The bearing of these facts has been so fully discussed for *Lycopodium* that no general application of them to the other genera need be repeated here, for the arguments and conclusions would be virtually the same.

EQUISETACEAE.

In other Pteridophyta besides the Lycopodineae abortive spore-bearing organs are also found ; for instance in the Equisetineae. Bearing in mind the Calamarian strobili, it may be an open question whether the annulus at the base of the strobilus of *Equisetum* is really a transitional body between the vegetative sheath and the sporangiophores (see Scott, *Studies in Fossil Botany*, p. 61). But whatever view be taken on this point, the fact is that frequently smaller sporangiophores, with fewer sporangia, or even one only, may be found at the base of the strobilus ; this is well seen in *E. palustre*. Sporangia may also be found on the annulus, and a good series was figured by Milde¹, showing intermediate

¹ Beiträge z. Kenntniss d. Equiseten (1851), Pl. LV, Figs. 21-38.

conditions between sporangiophores and teeth of the sheath. Ridley¹ and Glück² also have figured cases for *E. Telmateja*. I have a specimen of this species showing a number of isolated small sporangia on the annulus. Such examples at the base of the strobilus have probably a similar bearing to those incomplete sporangia at the base of the Lycopod strobilus. A further point for comparison is found in those frequent examples of continuation of the apex of the strobilus into a vegetative shoot³. This may be put in relation with the fossil *Phyllothea*, which Solms describes as 'having its fertile spikes repeatedly interrupted by ordinary vegetative whorls⁴.' Such facts tend to show that in the Equisetineae also the strobilus is not absolutely marked off from the vegetative region, while the proliferous *Equiseta* remind us of the *Selago* condition of *Lycopodium*. The facts are, however, not sufficiently distinctive to bear any considerable weight of argument, and some of the examples are plainly abnormalities.

OPHIOGLOSSACEAE.

In *Ophioglossum vulgatum* a rudimentary spike is often to be seen as a small peg-like growth in the place where a normal spike would be inserted. It is represented in Rabenhorst's Kryptogamen-Flora, iii, p. 537, Fig. 175 A. Similar abortive spikes have been seen in *O. reticulatum* and *pendulum*. Such cases show that leaves ostensibly sterile are potentially fertile. In *Botrychium Lunaria* extraordinarily small plants are found to bear fertile leaves, with the fertile segment proportional to the sterile. But in some cases of small weak plants the fertile segment appears to be entirely absent. Here again the case is similar to that in *Lycopodium*; and it can hardly be doubted that frequently the leaves when apparently sterile were in their first steps potentially fertile;

¹ Journal of Linn. Soc., vol. xx, p. 47.

² Glück, Sporophyllmetamorphose: Flora, 1895, Pl. V, Figs. 4-6, Text, p. 364.

³ For most of the species a 'forma prolifera' has been described. See Milde, loc. cit.; also Rabenhorst, Krypt.-Flora, iii; see also Ridley, loc. cit.

⁴ Fossil Botany, English Ed., Oxford, p. 181, Fig. 17.

in fact that here again potential fertility is more extensive than that which is realized. At least those leaves which bear undeveloped rudiments may be designated sterile sporophylls, and the imperfect spikes are to be regarded as vestigial, each being subtended not by imperfect, but by a fully developed sterile lamina¹.

FILICINEAE.

In the larger leaved Pteridophyta examples of incompletely developed sporangia or sori are not uncommonly met with; but owing to their being distributed over the large leaf-area they are less susceptible of theoretical treatment than in the simpler cases of the smaller-leaved forms. Many instances of transition from sterile to fertile leaves, or parts of leaves, in Ferns have been described; the balance of vegetative and reproductive regions, even on the individual leaf, shows some interesting analogies with that on the whole plant in some species of *Lycopodium*. Glück² has brought together a large number of such cases, intermediate between sterile and fertile leaves, which are so far interesting as they show the intimate relation of sterile and fertile leaves; developmentally it hardly needs to be reiterated that foliage leaves and sporophylls of Ferns are alike; the two types of leaf are merely variants of the same category of parts. Nor does it require to be stated again at the present day that in the individual the foliage leaves commonly precede the sporophylls. All the facts stated by Glück may be accepted as consistent with either the view that sporophylls are phylogenetically 'modified' foliage leaves, or that foliage leaves are sterile sporophylls. Stripped of all accessories, his conclusion, that all sporophylls are altered foliage leaves, is founded on the assumption that the development of the individual is a correct index of the evolution of the race, quite irrespective of the results of comparison. He arrives at an obvious ontogenetic conclusion, and states it as a phylogenetic truth.

¹ Dr. Lang informs me that abortive fertile spikes are commonly found also in *Helminthostachys*, subtended in each case by a fully developed sterile lamina.

² Flora, 1895, Heft 2, pp. 322-355.

For comparison with those species of *Lycopodium* which are fertile to their base, it may be recalled that at least one Fern exists in which the leaves are all fertile. Prantl records 'the remarkable fact that in *Lygodium subalatum*, not only are normal leaves fertile to the very base, but also the sub-primordial leaves, and even the primordial leaves already bear sorophores. So that fully sterile leaves, or primary and secondary segments in this species are as yet unknown¹.' Now it is to be noted that *Lygodium* belongs to a peculiarly ancient stock, which gives a special significance to the fact recorded by Prantl. And here it may be remembered that Prantl firmly held the idea of the priority of fertile over sterile leaves, and asserted it repeatedly². Though no one would now subscribe to his comparison of the Hymenophyllaceous sorus with the Moss-sporogonium, his other points should not for that reason be discounted. But however correct his opinions may be, the evidence in the case of Ferns is likely to be less conclusive than for Lycopods. For it seems to be true that rudiments of sporangia are most frequently preserved where the sporangia are borne singly, and make an early appearance on the part which bears them, as in the Lycopods; while in the Ferns the sporangia are formed relatively late upon the sporophyll, and usually in large numbers.

And here I may remark that the Ferns should not be taken as a general guide to the morphology of other Pteridophytes; they are clearly a very specialized series, and on that account have not been put in the forefront of the present discussion. The argument derived from comparison of species of *Lycopodium* stands upon its own footing, and requires neither support nor check from comparison with the larger-leaved Filices, with which they have no near affinity.

IMPERFECT SPORANGIA IN FOSSILS.

The facts being as above stated for living species of Pteridophytes, the question arises whether there is any similar evidence from fossils. It may be said at once that the facts are scanty, and the arguments to be based on them inconclusive.

¹ Schizaeaceae, p. 14.

² Hymenophyllaceae, p. 62; Schizaeaceae, p. 6.

In the genus *Lycopodites*, whatever errors may have been made in ascribing to it forms which are of other affinity¹, there are certainly some specimens which show a habit very like some of our modern species of *Lycopodium* and *Selaginella*, and with their strobili but little differentiated from the vegetative region. *Lycopodites ciliatus*, Kidston², from the middle coal measures, shows no clearly differentiated strobilus: the sporophylls are quite like those of *Selaginella*, with ciliate margin and broad base, subtending sporangia which are not radially elongated; but there is no evidence of any alternating sterile and fertile zones, as in *L. Selago*. Mr. Kidston remarks that 'mixed with the leaves are macrospores of small size.' These are well seen, and of the zonated type: probably the plant was heterosporous, and may have been like a *Selaginella* with an ill-defined strobilus. Goldenberg³ distinguished two divisions of *Lycopodites*: '*Pananthites*,' in which the sporangia are sessile in the axils of leaves not clearly differentiated from the foliage leaves, and '*Lepidotites*,' those in which the sporangia are seated in terminal strobili. A good example of the latter is *L. Stockii*, Kidston, quoted by Solms-Laubach as having the habit of *Lycopodium Phlegmaria*⁴. These and other cases afford sufficient evidence that both of these types existed at least as early as the Middle Coal Measures. But as yet I know of no early evidence of the 'Selago' condition in them: it is to be remarked, however, that specimens of these small fossils are rare, and that they are easily overlooked.

Various *Lepidostrophi*, in which the strobilus is definitely limited, show imperfect sporangia towards the apex. In *L. Brownii* I have observed and figured sporangia of small size towards the upper end of the strobilus⁵: they contain no

¹ Fossil Botany, Solms-Laubach, Engl. Ed., p. 186.

² See Kidston, Trans. Nat. Hist. Soc., Glasgow, 1901, p. 37. Mr. Kidston has kindly shown me his specimen (No. 1743), upon which, together with his text, these notes are based.

³ Flora Saraepontana Fossilis, 1855, p. 9; see also Kidston, loc. cit., p. 32.

⁴ Fossil Botany, Engl. Ed., p. 186.

⁵ Studies, i, Phil. Trans., vol. 185, p. 527, Pl. XLVIII, Figs. 95, 99, 100.

spores, and from their general characters seem to be arrested sporangia, rather than young ones in the normal course of development: but it is difficult to arrive at certainty on this point. The base of this cone is unfortunately incomplete. Dr. Scott quotes also a case of a *Lepidostrobus* in his own collection, in which the sporangia 'at the top are small, still closed, and with thick walls, showing that development has been arrested before the absorption of the inner layers.' 'I find the same thing,' he says, 'in my new cone, *Lepidocarpon Lomaxi*, as well as in a microsporangiate cone, which may probably belong to the same species¹. It seems more common to find the apex of the cone complete, than the base. One specimen in Mr. Kidston's collection (slide No. 98) of *Lepidodendron Veltheimianum*, from Pettycur, shows a gradual lessening of size of the sporangia towards the base, but no extreme reduction to vestigial proportions was observed. In *Spencerites insignis*² a section of the cone 'which appears to have been made near one extremity of the strobilus,' shows some sporangia of small size: these have been re-examined and described by Scott³, who, without being able to locate them at the apex or base of the cone, regards them as being 'abortive organs' the development of which has been 'arrested at a rather early stage.' In *Sigillaria* the cones, which are probably heterosporous, are usually borne on long pedicels: the bases of these strobili have been examined in a number of specimens in Mr. Kidston's collection: they show gradual transitions from the broad ciliated sporophylls, to the smaller, narrower basal bracts: there is no evidence of abortive sporangia, which indeed could hardly be expected in specimens which are all impressions. But the similarity in external appearance to large strobili of *Lycopodium* or *Selaginella* is sufficiently close to raise the impression that they probably existed.

Speaking of *Calamostachys Binneyana*, Williamson and

¹ Extracts from a letter from Dr. Scott.

² Williamson, Fossil Plants of the Coal Measures, ix, Fig. 53, p. 341.

³ 'On Spencerites,' Phil. Trans., vol. clxxxix, B, 1898, p. 93, Pl. XV, Fig. 15.

Scott¹ in discussing certain sporangia which are filled with parenchymatous tissue, express the opinion that they are 'more or less completely abortive sporangia.' But as their position in the strobilus is uncertain, they have no direct bearing on the question under discussion in this paper. And here may be mentioned that curious fossil *Phyllothea deliquescens*, Goepp.²: it seems uncertain what may be the true interpretation of it, though Solms speaks of the genus as differing from true Equisetaceae in having its fertile spikes repeatedly interrupted by ordinary vegetative leaf-whorls. This, however, cannot be closely compared with the 'Selago' condition of Lycopods.

Lastly, there is the case of *Cheirostrobos*: Scott, in his monograph on this fossil³, worked from two specimens: one probably a peduncle, the other a more complete cone. The latter shows clearly a reduction in size of the basal sporangia and sporophylls. This is well seen in the section figured as Scott's Photograph 15, and drawing 15, in which the lowest sporangium is barely one quarter the length of one of those at the middle of the cone: the whole outline of the cone is oval at the base, owing to the gradual diminution in length of the sporophylls and sporangiophores. On the peduncle⁴ the bases of the leaves or bracts are present, showing superior and inferior lobes: there is evidence, however, that the latter are smaller and simpler in structure than the corresponding lobes which are really fertile. As the foliage of the plant is unknown, it is impossible at present to base definite conclusions on these facts, beyond recognizing the reduction of the sporangia, and of the 'fertile segment,' at the base of the cone, and on the peduncle.

The general results to be drawn from these fragmentary facts from fossils are, that the strobili of fossils were not always definitely marked off from the vegetative region, and

¹ Phil. Trans., 1895, vol. clxxv, B, p. 910; also Williamson, Phil. Trans., 1880, Pl. XVI, Fig. 18.

² Solms-Laubach, Fossil Botany, Engl. Ed., p. 181, Fig. 17, B.

³ Phil. Trans., vol. clxxix, B (1897), p. 1.

⁴ Loc. cit., p. 17.

that the strobili are, when defined, similar in their broad characters to those of modern times, showing diminution of sporangia at the apex, and also at the base. Though there is thus little conclusive evidence from such facts as have been brought forward relating to fossils, at least we may assert that, so far as it goes, it does not run counter to the results that have been obtained by comparison of living plants.

GENERAL CONCLUSION.

Abortive parts are not such prominent features in plants as in animals, and they have played a less important rôle in the theories of vegetable morphology than in those of comparative zoology. This is probably to be understood as a consequence of the continued embryology of plants, and the unlimited number of the appendicular parts, arising from their persistent meristems. Thus the initiation of individual parts, such as the reproductive organs of the sporophyte, may be deferred so late as to remove them far from the initial embryology of the plant. In animals, on the other hand, it is frequently the parts initiated early in the embryology which supply the best examples of vestigial organs.

But in the above pages we have seen that incompletely developed parts are frequent among the Pteridophyta. Such recurrent phenomena must not be ignored. Their treatment should be consistent with that of similar phenomena in other groups of plants, or in animals. And naturally we turn to the Phanerogams for guidance in morphological method, since in their comparative treatment abortive parts have been taken fully into account. It will be unnecessary to quote special instances of this, for the recognition of certain abortive parts as vestigial—that is representing parts which in the ancestry were once actually functional—is a common position in floral morphology. The ready acceptance of such conclusions for Flowering Plants is probably due to the fact that, in many cases, closely related genera and species allow of a complete and consecutive train of comparison, while also the arguments have for the most part dealt only with the parts of the flower,

and have not touched the broader and more contentious question of the relations of the vegetative and reproductive parts.

On the other hand, as Goebel has pointed out, all incompletely developed parts are not to be assumed to represent parts which have been functional in the ancestry; and in dealing with the Pteridophytes, as also with Flowering Plants, we must distinguish between those parts which are really arrested and vestigial, and those which are simply supernumerary primordia. It is on a basis of comparison, combined with examination of the individual part, that this distinction can be drawn: such comparison in the large living genus, *Lycopodium*, has led to the view that the arrested sporangia at the base of a strobilus are really vestigial, while those at the apex may be simply supernumerary.

Perhaps for our present purpose the best analogy with this is to be found in Prantl's theory of the honey-leaves in the Ranunculaceae¹. Prantl showed that the glandular honey-leaves are, on the grounds of form, position, and development, to be taken as stamens, which in place of forming pollen-sacs, developed usually as honey-secreting structures: they are in fact sterilized sporophylls. Their appearing prior to the stamens in the ontogeny does not prevent the floral morphologist from admitting their derivation by metamorphosis from stamens, which in their fully developed state are formed later in the individual flower. We may leave on one side for the present the wider question raised by Drude² whether other parts of the flower also may not be metamorphosed stamens: on Prantl's theory of the honey-leaves an analogy is provided for us, which fits the present view for the sporophylls and sterile foliage leaves of *Lycopodium*: in both cases it is suggested that, by functional and structural modification, a series of parts differing in function from the original type has been intercalated: and that though these appear earlier in the individual, they were of later origin in the descent. It is thus attempted in this paper to apply to the strobiloid Pteridophyta the same method as is accepted in the morpho-

¹ Engler's Jahrb., vol. ix, p. 225.

² Schenk, Handbuch, iii, 2, p. 302.

logy of a family of Flowering Plants. And just as the honey-leaves may be altered stamens, so may the foliage-leaves of Lycopods be sterilized sporophylls.

But, conversely, we may turn for analogy to the Bryophyta. A comparative treatment of the sporogonium illustrates the progressive sterilization and intercalation of a vegetative phase between fertilization and spore-production. In the above pages the analogy has been pointed out between the balance of vegetative and reproductive phases in the sporogonium of certain Mosses and that in the Lycopods; and how in both series it may be recognized that there is a reproductive zone characterized at its lower limit by structural evidences of *evanescent* spore-development, and at its upper limit by evidences of *nascent* spore-production; in both regions the development being incomplete. The idea is thus presented of a fertile phase in the individual, which may in the descent move on to later phases in the individual, progressive sterilization taking place below, and progressive apical growth extending the plant above; with the result of an increasing vegetative system for nutrition. This analogy in the two large series should be allowed due comparative weight, and not discounted on the mere ground that the Bryophyta have a concrete archesporium and no appendages, while the Lycopods are leafy and bear separate sporangia. The lines of descent have probably been quite distinct; but the demands of nutrition, which dominated both, were alike.

The endeavour must be made to preserve a consistent morphological method for the Pteridophyta and for other plants both higher and lower in the scale. And to this end, just as much prominence should be given to the abortive parts which are present in the Pteridophyta, and especially in the simple Lycopods, as to the abortive parts in the Angiospermic flower: and they should be open to the same interpretation. On the other hand, the progressive sterilization admitted as an important factor in the progress of the Bryophyte sporogonium should equally be admitted, where the facts indicate it, in the upward progress of the Pteridophyta.

Following then the methods of comparative study of Bryophytes and of Angiosperms, and employing them upon the results yielded by specific study of the genus *Lycopodium*, there seems only one reasonable reading of the facts: that sporangia, previously in the race fully formed, have become abortive; either partially, so as to leave vestigial traces, or entirely: that sporophylls have thus passed to the condition of foliage leaves: and that thereby the vegetative system has been increased. There seems further a reasonable probability that, in *Lycopodium* at least, the whole of the foliar system may have originated in this way¹.

It was remarked above that the general line of argument in this paper would follow naturally, perhaps we may even say necessarily, from the acceptance of the theory of antithetic alternation. But it is also consistent with the view that the alternating generations are really homologous, if spore-production appeared early in the history of the neutral generation²: and this it must certainly have done, if the earliest sporophytes were like those simpler ones we see at the present day. Comparison leads decisively to the conclusion that the formation of spores was not a happy after-thought, imposed upon an already present and extensive vegetative system, but of early occurrence in the primitive individual. It may or

¹ This idea has already been put forward by Naegeli in the following sentences (Abstammungslehre, p. 477): 'Die zweite Stufe ist also ein unverzweigter beblätterter Stengel; die noch höchst einfach gestalteten Blätter sind alle gleich und sporogonientragend: . . . in der Abstammungslinie der Lycopodiaceen mag diese Stufe grosse Aehnlichkeit mit einem unverzweigten *Lycopodium Selago* gehabt haben.' Though I should not subscribe to the exact mode of origin of such a form suggested by Naegeli, it is to be remarked that he clearly contemplated an organism in which all the leaves are originally sporophylls, and that the vegetative system originated by deferring the spore-production to later stages of the individual. The presence of the imperfect sporangia at the base of the fertile region in so many Lycopods supplies important evidence from vestigial organs, which coming after the enunciation of his views based on other grounds, is a very strong support of them.

² Compare Coulter, Origin of the leafy Sporophyte, Bot. Gaz., 1899, p. 46. He suggests a possible antithetic origin in Bryophytes, and homologous for Pteridophytes; in the latter case he supposes a leafy axis bearing spores, derived from the thallus (p. 55).

may not have antedated the whole vegetative system of the sporophyte, as the antithetic theory implies: but in any case spore-formation was of early date.

The theory of sterilization of sporophylls above stated for Lycopods embodies a tangible idea, which is in accordance with the facts, including those relating to abortive parts. It was met on a previous occasion by Prof. Goebel, first by a reference to large-leaved forms, and especially to his earlier observations on the sporophylls of *Onoclea*; but without reference to the detailed facts for *Lycopodium*, on which my argument was based¹; subsequently by a simple 'non possumus'; and here again there was no reference to the facts relating to *Lycopodium*²; nevertheless he remarks that 'Botany as a science is more concerned with facts than with phylogenetic theories.' He concludes, after a reference to Ferns, but still ignoring the Lycopods, that 'as to what occurred in prehistoric ages, we require far more convincing proofs than are afforded by the materials at present within our reach, before we can speak with any assurance.' It was met by Dr. Glück by the old assumption³, that the ontogeny is a key to the phylogeny: that because the foliage leaves are prior in the individual, therefore the later-formed sporophylls are altered foliage leaves. Readers may then choose between, first, the 'non possumus' position, which declines to entertain any phylogenetic theory on the point in question: I think this will not commend itself to inquiring minds, if they give attention to the facts which I have brought forward. Secondly, the position which assumes a prior vegetative system of the sporophyte, but gives no explanation how or when, in the descent, the spore-production, which is so constant a feature at the present day, came to be imposed upon it⁴. And thirdly a coherent

¹ Annals of Botany, vi, 1892, p. 359. Also Ber. d. d. Bot. Ges., 1887, p. 69.

² Science Progress, iii, p. 120.

³ Sporophyllmetamorphose, p. 383.

⁴ Goebel wrote as follows on this point (Ber. d. d. Bot. Ges., p. 74): 'Die Pflanze bildet nur eine Art von Blattanlagen, und zwar Laubblattanlagen, von denen aber, infolge bestimmter Einwirkungen, regelmässig ein Theil zu Niederblättern, ein Theil zu Sporophyllen sich ausbildet, während die übrigen sich als Laubblätter weiter entwickeln.'

hypothesis, which fairly meets the facts, and proceeds on the same methods with regard to vestigial organs as is adopted in floral morphology. Goebel, alluding to my previous paper, says that my views were based on 'phylogenetic grounds¹.' These certainly came in, but the actual foundation was on the fact that incomplete sporangia exist at the base of the strobilus². Whatever decision is now arrived at, it will have to take into account those imperfect sporangia at the base of the fertile region in Lycopods, and elsewhere, which, on grounds explained above, I regard as vestigial. If these are vestigial, then the position taken up in this paper is the natural consequence. If they are not vestigial, what are they?

Postscript.—Mr. Kidston, by letter, calls my attention to the case of *Sphenophyllum majus*, Bronn (see Kidston, Trans. Nat. Hist. Soc., Glasgow, vol. vi, p. 128), in which the cone is little modified in form from the ordinary foliage branch. The internodes are not shortened, and the bracts not more reduced in the limb than is seen in the segmented leaves of the ordinary foliage branches. Specimens of this fossil come from the Middle Coal Measures, and thus the similarity of the foliage shoot to the strobilus is seen in another fossil form of early date.

F. O. B.

¹ Science Progress, iii, p. 120.

² See Studies, i, Phil. Trans., 1894, B, p. 535.

Observations on the Biology and Cytology of *Pythium ultimum*, n. sp.

BY

A. H. TROW, D.Sc., F.L.S.

—♦—
With Plates XV and XVI.
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FOR some years a detailed knowledge of the cytology of the genus *Pythium* has been a great desideratum. Notwithstanding the attention which has been bestowed on the Phycomycetes, and especially on the Oomycetes in recent years, and the great interest which has been excited by the results achieved, this very interesting genus has been practically neglected. We need only to refer to the work of Wager ('89, '96, and '00) on *Peronospora* and *Cystopus*; Berlese ('97) on the same and other genera; Stevens ('99) and Davis ('00) on *Albugo* (*Cystopus*); Istvanffi ('95) on *Cystopus*, *Saprolegnia*, and other genera; Humphrey ('92), Hartog ('95), Dangeard ('90), and myself ('95, '99) on *Saprolegniaceae* (chiefly *Saprolegnia* and *Achlya*); and Lagerheim ('00) on *Monoblepharis*, to show how rapidly our knowledge of the cytology of the Oomycetes has grown in the last decade of the nineteenth century. Little, however, has been done for the genus *Pythium* since the time of Pringsheim and De Bary ('87), to whose fundamental work it is no longer necessary for us to refer at length. Ward ('83) alone in this country seems to have paid attention to the genus, and his

[Annals of Botany, Vol. XV. No. LVIII. June, 1901.]

observations are practically confirmatory of those already made by De Bary. Although the microscope has been considerably improved during the last twenty years it is only just to say that the most recent observations on living material add little to the knowledge handed down to us by the older observers.

The only observations of importance on the karyology of *Pythium*, founded on the examination of fixed and stained material, are those of Fisch ('85) and Dangeard ('92).

Fisch states: 'Im jungen Oogonium, vor der Oosphärenbildung, sind ziemlich regelmässig 10–20 Zellkerne anzutreffen. Bei der Bildung der Oosphäre rücken sie zusammen, bis sie dicht aneinander liegen, und verschmelzen dann zu einem einzigen ziemlich grossen Eikern. In der Antheridialzelle habe ich immer nur einen Zellkern gefunden, bezweifle aber nicht, dass auch mehrere vorkommen können, die aber dann sicher vor der Befruchtung zu einem einzigen verschmelzen. Der Zellkern der Antheridialzelle wandert mit dem Gonoplasma in die Oosphäre über und verschwindet hier mit dem Eikern.' At the time Fisch wrote this it appears to have been quite the fashion to assume that a multinucleate organ became uninucleate by a process of wholesale nuclear fusions. The process of fusion was often definitely described, as by Fisch in the case before us. Careful observations have, however, shown that in many cases at least, e.g. *Vaucheria*, *Peronospora*, *Cystopus*, *Saprolegnia*, and *Achlya*, no such fusions are demonstrable, and that the uninucleate condition is reached in quite a different fashion. It is not difficult for the experienced botanist to realize how such errors of interpretation can arise. Even with the most refined of the modern methods it is often difficult to get thoroughly satisfactory proofs of the behaviour of the nuclei in any one case. Wager ('00) has indeed felt himself in a position to question the tenability of Fisch's view. He says, 'It is probable that the mode of fertilization described by Fisch for *Pythium* will be found to be untenable.' His view is a just one, but it is as well to bear in mind that great differences in the karyology

of species of the same genus (as at present defined) may occur, as has been well shown recently in the case of *Cystopus*.

It would be interesting to know the exact meaning attached by Fisch to 'verschwindet' in the last line of the quotation. For nuclear fusion he uses the verb 'verschmelzen,' which is the usual term. It is noteworthy that in bad preparations the male and female nuclei are very difficult to demonstrate. They are apt to disappear from sight altogether. It is possible that Fisch never really traced in *Pythium* the nuclear fusion characteristic of fertilization. It is certain that in one species of *Pythium* at least no such fusions as those described by him take place.

Dangeard ('92) says: 'Le *Pythium proliferum* possède également de nombreux noyaux, soit dans les oogones, soit dans les anthéridies.' The observations recorded in this communication show that Dangeard's view, which so far as it goes does not materially differ from that of Fisch, is a correct one.

My work on *Saprolegnia* and *Achlya*, especially that part of it which dealt with the mode of fertilization and the degeneration of nuclei, made me anxious to study some member of the Oomycetes in which there was no question as to the actual sexuality, and where moreover one could follow the course of degeneration in indubitably degenerate nuclei. The Peronosporaceae offered the right kind of material.

Functional sex is universally admitted to be characteristic of this group. The periplasm might be expected to furnish the degenerate nuclei. That the genus *Pythium* is, as Fischer ('92) points out, a difficult one, with a considerable number of 'critical' species, in part at least, very badly defined; that nevertheless it has been selected to furnish type plants in two well-known and highly esteemed textbooks; that its study has been specifically introduced into more than one syllabus of elementary botany (or biology); and above all, as already pointed out, that a detailed knowledge of its karyology was still a great desideratum—furnished ample motives for its selection as the subject of a thorough investigation.

Most botanists engaged in actual teaching are probably familiar with certain difficulties which attend the practical study of the genus. In my own experience *Pythium de Baryanum* was commonly met with on dying cress-seedlings a few years ago. It is said that the spores of this species are universally present in the soil of gardens. Be that as it may, it has not put in an appearance at Cardiff during the last four years, notwithstanding repeated (annual) efforts made to secure it. *Pythium de Baryanum* is apt to be displaced by allied forms, and these are, as a rule, much less suitable for teaching purposes. In fact, many of them, owing to the delicacy of their mycelium and the irregularity with which the reproductive organs make their appearance, are almost, if not quite, useless for the purposes of demonstration to beginners. Even with so good a type as *P. de Baryanum* young students find it most difficult to make passable fresh preparations from infected cress-seedlings. It is not generally recognized, however, that the genus possesses a number of species which are pure saprophytes, and that of the remainder many can be readily cultivated as saprophytes. The parasitic species offer for most purposes no advantage over the saprophytic ones, for there is little to interest us in the attack of the parasite on its host in this case. Indeed in many species of *Pythium* the parasite apparently attacks its living host in just the same way that the saprophyte attacks the dead organic matter. The saprophytic species are, however, much easier to work with than the parasitic; for (1) dead organic matter is more easily procurable than living organic matter in the form of seedlings, and (2) suitable dead organic matter can easily be sterilized, while it is very difficult, if not impossible, to sterilize such objects as living seedlings, and (3) dead organic matter can be kept in the sterile condition for long periods with ease. So great are the advantages that teachers would do well to discard the haphazard methods now in vogue of securing cultures of parasitic species (frequently mixed), and provide themselves with a pure culture of a suitable saprophytic species, from which at any time

abundant supplies of material could be obtained for demonstration.

The method adopted by me to secure material for investigation was the well-known one of growing seedlings in the presence of excess of water, both in the soil and in the air. Garden soil was obtained from various sources in the neighbourhood of Cardiff and sowings of cress made in it in the usual way. The seed pans were kept in a closed cold frame, which was drenched with water twice a day. Most of the seedlings, as they came up, of course 'rotted off,' and furnished the *Pythium* material. Somewhat to my surprise all the seedlings did not perish. It would be interesting to know the reason. The parasitic species of Fungus which presumably caused the death of the seedlings were very unsuitable for further examination. The mycelium was very delicate, and the reproductive organs were found either in small numbers or were conspicuously absent. *Pythium de Baryanum* was not seen. Some of the most suitable species were transferred to Petri dishes and cultivated on fresh seedlings in abundance of water. The result was disappointing. No single suitable species was secured.

In July, 1899, however, a very rotten cress-seedling, which was lying prone on the saturated soil, was taken for examination. The stem was found on careful teasing to contain numerous spores. The spores were transferred to a cover-glass and a moist chamber culture set up, using for nutrient material a fragment of boiled cress cotyledon. Germination took place at once, and a good culture was obtained. This was transferred to a Petri dish and a larger culture made on two or three boiled cress-seedlings. Growth was luxuriant: in a few days a fine mycelium filled the dish and before the end of the week there were numerous sexual organs present and even ripe oospores. This culture furnished the material for all the subsequent ones. Further moist-chamber cultures were set up, using a single spore as the starting-point, and the life history was worked out in detail from the germination of the asexual spores ('conidia,' as we had better call them)

and oospores to the formation of new conidia and ripe oospores.

In August, 1900, it appeared advisable to attempt to secure a really pure culture, i. e. not simply a culture of a single species of *Pythium*, derived from a single spore, but a culture which in addition was free from all other organisms whatsoever. Two or three species of small Amoebae which multiplied by division and encysted with the greatest regularity in the moist-chamber cultures, some Infusoria and Monads were got rid of by setting up a series of moist-chamber cultures. A culture, while still fresh and vigorous, was thoroughly washed with sterilized water and a small fragment used to inoculate a second one, the process being repeated a considerable number of times. Bacteria however obviously still remained, and it was necessary to find some other means of getting rid of these. The method used by Klebs to isolate *Saprolegnia mixta* was tried and much time consumed in the process, but to no avail, for the Bacteria flourished on the nutrient gelatine, and the *Pythium* made no growth at all. An attempt was next made to check the growth of the Bacteria by means of acids. A culture on sterilized cress-seedlings in a Petri dish was set up, using a 1% solution of acetic acid instead of water. No growth of any kind took place in this, either of Bacteria or *Pythium*. Before proceeding to test the effect of other organic acids and weaker solutions, an experiment was made by setting up a culture on the acid leaves of rhubarb. Very fine cultures were produced in this way practically free from Bacteria. But for a fortunate and almost accidental observation, I should no doubt have spent much time in making and maintaining a pure stock culture by the use of rhubarb leaves. In cultures of the Fungus on bits of cabbage leaves, parallel to those on rhubarb leaves, it was noted that if the leaf projected above the surface of the water in the Petri dish, the mycelium in very luxuriant cultures developed a slight aerial growth. It was obviously possible to exaggerate this by favourable conditions, and, by means of cuttings taken from it, obtain the pure infecting material that

was required. Accordingly chunks of potato, carrot, and turnip, paste made of starch, flour, pea-meal, and various other materials, were sterilized in suitable glass pots and inoculated with the relatively pure material obtained from the rhubarb leaf cultures. On the second day after inoculation there was a growth of pure white aërial mycelium on potato, carrot, and turnip a centimetre in height, which on the following day had increased to almost an inch. The mycelium indeed quickly filled the whole pot. It was obvious that a very simple way of obtaining a pure culture had been found. It was only necessary to sterilize potatoes and inoculate them with a trace of the aërial mycelium. This was done, and the pure culture thus obtained provided all the material upon which the more important results were worked out.

Such pure cultures can be obtained, no doubt, with ease in many species of *Pythium*, and it is to be hoped that in the future the method described will be largely utilized.

It is perhaps of interest to add that the attempt to cultivate the Fungus on different kinds of paste yielded negative results.

OBSERVATIONS MADE ON LIVING MATERIAL.

As already indicated, this species of *Pythium* has been studied in three distinct types of cultures:—(1) Moist-chamber cultures, the nutrient material being generally the legs of house-flies; (2) Petri-dish cultures, the nutrient material being generally dead flies or bits of cabbage leaves; and (3) Glass pot cultures, the nutrient material being boiled potato.

The glass pots were cylindrical, 4 cm. high, 6 cm. in diameter, and of a capacity of about 100 c. cm. In the first two types the mycelium develops, with rare exceptions, entirely under water and so is aquatic; in the third the mycelium grows out into the air, and so is aërial. In all cases certain branches of the mycelium penetrate the nutrient substratum, and thus are intramatrical, but the greater portion of the mycelium is extramatrical. The intramatrical parts do

not differ from the extramatrical in any material way. Both may bear the reproductive organs. There is no differentiation of rhizoids or haustoria.

The details of the life history were worked out almost entirely with the help of the moist-chamber cultures. This was necessary not only as a means of determining the systematic position of the species, but especially as a guide to the proper sequence of the series of sections required to illustrate the karyology.

No attempt will be made to describe the results in the order in which they were obtained, but rather in the way in which they can be most easily followed, and in the order in which they could most quickly be verified.

Germination of the conidia. The conidia are either terminal and spherical or intercalary and barrel-shaped. If they are placed in distilled water they remain at rest for an indefinite period. If placed in a drop of cabbage water they germinate at once, one or more germ-tubes putting in an appearance with the greatest punctuality in about an hour's time. So regular is this that if thousands of conidia are sown at the same time it is generally impossible to find a conidium with a germ-tube three-quarters of an hour later, or a conidium without one after two hours. Germination likewise takes place if a fly-leg is added to a drop of tap-water containing conidia, but not quite so regularly. Figs. 1 and 2 (Pl. XV) fully illustrate the process in the case of two conidia which were placed in cabbage-water at 9.50 a.m. The germ-tube makes its appearance very suddenly, and its apex is filled with hyaloplasm. Sometimes, as in Fig. 1 *b*, an elongated vacuole makes its appearance, and then germ-tubes generally appear opposite its poles. In a few cases there have been observed faint indications of a segmentation of the protoplasm, as if zoospores were to be formed. No zoospores, however, have been seen at any time, and it may be regarded as certain that the conidia are no longer capable of producing them; for (1) there is no indication of a terminal beak such as is usual in sporangia, (2) no germination takes place in

distilled water, (3) tube-germination takes place, although slowly and irregularly, in material placed in running tap-water, (4) in suitable nutrient solutions each conidium may give rise to four or five germ-tubes whose place of origin is quite indefinite, and (5) empty conidia have never been seen, although very numerous experiments have been made on germination. There can be no doubt that in this species the conidium has completely lost its sporangial character. *Pythium* furnishes us with a series of forms—*P. megalacanthum*, *P. de Baryanum*, *P. vexans*, and *P. ultimum*, roughly parallel to the corresponding series in *Peronospora* described by von Tavel ('92), viz. *P. nivea*, *P. densa*, *P. Lactucae*, and *P. Radii*, in which the stages in the conversion of a typical sporangium into a conidium are preserved for us.

The conidium generally becomes vacuolated if kept in distilled water, or in exhausted nutrient solutions. They have been kept under observation for seven months. Whatever may be the age, germination takes place at once in appropriate nutrient solutions.

Growth of the mycelium. Growth is extremely rapid, as may be seen at once by reference to Figs. 1 and 2. The hyphae indeed, in a few hours, grow out of the drop altogether.

Branching is of course monopodial, but very irregular. In order to keep the same culture under observation for any period of time greater than twenty-four hours, it is necessary to remove the cover glass, and by suitable manipulation bring the wandering hyphae once more within the limits of a small drop of nutrient solution. The hyphae are so delicate that it is difficult to do this without causing serious injury. Under careless manipulation the protoplasm may be seen coursing down the older hyphae in the most violent way. Such injury however is not irreparable, for the protoplasm in the apical parts is fairly stable and not readily destroyed, and fresh growth takes place at once. In about two days in warm summer weather (day temperature averaging about 22° C.) the small drop is traversed in all directions by a net-

work of hyphae forming a felt-like mycelium, and the formation of the reproductive organs commences. These are of two kinds, asexual and sexual, of the type characteristic of the genus, and they appear in abundance in these cultures in the usual succession. We have first a crop of conidia and then a crop of oospores, the change from one type to another being quite gradual however, so that on adjacent hyphae conidia and oogonia may be seen in course of development at the same time.

Formation of the conidia. The end of a branch stops growing in length, but grows in breadth so as to produce a spherical head; this increases in size, and is finally cut off from the hypha which bears it by a transverse wall. Intercalary conidia are formed in a similar manner. The protoplasm of the conidia is about as dense as that of the young hyphae, and may or may not contain one or more vacuoles. Thus a conidium is simply a terminal or intercalary swollen portion of a hypha, cut off from the rest of the mycelium by one or two transverse walls.

Formation of the sexual organs. An oogonium is at first exactly like a conidium. Even when it is cut off from its supporting hypha by a transverse wall, it requires some experience to recognize it. It appears of a darker colour, due, no doubt, to the reserve materials present, but this is not apparent to the uneducated eye. Even a novice, however, is able to recognize an oogonium when it is accompanied by an antheridial branch or an antheridium. Fig. 3 gives the result of a study of the formation of the sexual organs up to the complete development of the antheridium. The observations commenced at 9.20 a.m. upon the two young oogonia shown in Fig. 3 *a*. The upper of these alone reached maturity; the protoplasm of the lower and younger was used up to provide the material for the development of the older. In undisturbed cultures in Petri dishes this never happens, so that the anomaly is no doubt due to the necessary preliminary manipulation of the preparation. In four hours the oogonium had grown to its full size. At about 2.5 p.m. the antheridial

branch suddenly made its appearance, immediately below the oogonium, and thirty minutes later, as shown in Fig. 3 *e*, the antheridium was fully formed. Nearly the whole of the remainder of the protoplasm in the aborted oogonium was used up in the formation of the antheridium. It is noteworthy that the oogonium occupies a terminal position until the antheridium is formed, when it is forced into a lateral one, a phenomenon common enough in cymose growths of very different kinds. Such displacements, combined with eccentricities of behaviour induced by the manipulation of the preparations, make continuous observations very tedious, and often practically impossible. The details of fertilization cannot be followed in organs orientated as in Fig. 3 *e*. Fig. 4 gives the result of a study of the maturation of the sexual organs and of the process of fertilization. The oogonium represented in Fig. 4 *a* was placed under observation and watched until, at about 7.55 a.m., the antheridial branch made its appearance. It was kept under constant observation until 2.0 p.m. and drawings made at frequent intervals. It would serve no useful purpose to describe in detail the changes observed from time to time in the protoplasm, for we appear to have no clue to their significance. It would be just as unprofitable as to describe the different shapes assumed by an *Amoeba*. The figures represent all that is of interest in the present state of our knowledge. We note that the appearance of the protoplasm in the oogonium changes considerably during the first three hours. Denser patches appear and disappear. The differentiation of the oosphere does not take place in the manner described by De Bary for *Pythium gracile*, or by Ward in the species investigated by him. Further, there is no differentiation of gonoplasm and periplasm in the antheridium.

The denser protoplasm, including all the particles of reserve material, migrates towards the centre of the oogonium, leaving behind for a time an inconspicuous, faintly granular layer between it and the wall of the oogonium, as shown in Figs. 4 *e* and 4 *f*. De Bary and his followers regarded the

dense central mass as the egg, and the clear peripheral layer as the periplasm. The central mass is in continuous movement, but tends to round itself off more and more, though it never acquires a smooth outline like that of the eggs of the Saprolegniaceae. Moreover the delicate peripheral layer completely disappears as represented in Fig. 4g, so that if we accepted the current view as to the significance of the central mass, we should have to admit that the periplasm disappears during the differentiation of the egg. The warted appearance of the central mass is very characteristic of this stage, and is apparently due to an accumulation of reserve globules. The study of sections proves what the examination of the living material leads one to suspect, that the central mass includes both egg and periplasm, and that the egg is completely masked by a dense mantle of periplasm containing globules of reserve material.

Turning our attention to the antheridium, we note that its growth is rapid, and that after a time two or three granular aggregations make their appearance in it. These *may* be nuclei, but it was impossible to furnish proof of their real nature. As the central mass begins to form in the oogonium (see Fig. 4e), a fertilization-tube becomes apparent, connecting the central mass with the antheridium. This *gradual disclosure* of the fertilization-tube has been generally interpreted apparently as its *growth*. There can be little doubt that it was formed before the differentiation of the oogonial contents began, as I have never been able to get a view of its apex, and have thus never been able to trace its growth. Different species of *Pythium* may behave differently in this respect. Certainly De Bary's figure of *Pythium gracile* suggests that such is the case, and the point thus deserves further investigation in other species.

Fertilization. The question of fertilization is a curious one. It seems generally admitted that the process of fertilization can easily be followed in this genus. Authors, however, apparently mean by this that they can trace a *slow migration* of the protoplasm down the fertilization-tube.

This slow movement may last for from one to two hours according to De Bary. In Fig. 4*g* two granules are represented in the fertilization-tube. They slowly passed on down the tube and necessarily disappeared from sight before they reached its end, that being concealed from view. In Fig. 4*h*, the progress made in the emptying of the antheridium is scarcely noticeable, while in Fig. 4*j*, drawn half an hour later, it is practically completed. The actual process of fertilization may have taken place at any time between 1.0 p.m. and 2.0 p.m. It almost certainly takes place quickly. What the significance of the gradual emptying may be it is difficult to say. Some evidence has been collected to show that much of the antheridial contents pass into the periplasm. On three occasions a little mound of protoplasm collected around the indentation made by the fertilization-tube and streamed away over the central mass from that point. It is significant that the egg rounds itself off and appears covered with a membrane before the last traces of protoplasm leave the antheridium. The last visible particles, as in Fig. 4*j*, do not pass out as such. They appear to be dissolved *in situ*, and so slowly vanish.

Observations on the living material do not suffice to prove the existence of fertilization, much less the mode in which it is effected. We have in fact the usual difficulty to contend with, the large female gamete is too dense to allow us to watch the entry of the small male gamete. It seems abundantly clear, however, that we have no justification for recognizing a differentiation of the contents of the antheridium into gonoplasm and periplasm. A study of stained sections is absolutely necessary for the proper interpretation of the phenomena observed in living material.

Maturation of the oospores. The maturation of the oospores may be completed in about twenty-four hours after fertilization has taken place. Three points of interest have to be noted. The original thin membrane covering the young oospore increases considerably in thickness by the deposit on the inside of a thick layer of some kind of cellulose. The

wall becomes distinctly two-layered, and as it grows older it acquires a yellowish colour. The so-called oil globule, better named reserve globule, develops in the middle of the protoplasm, and lastly between the central globule and the wall a large nucleus makes its appearance. Fig. 5 shows successive stages in maturation in the case of an oospore which ripened very slowly. The egg was fertilized on 29/8/99 at about 5 p.m. The oospore took more than three days to ripen.

The following summary will serve to show roughly the time occupied by this species of *Pythium* in passing through the various stages of development :—

For the growth of the mycelium	48 hrs.
For the completion of the development of a half-grown oogonium, reckoning up to the first appearance of the antherial branch	4 $\frac{3}{4}$ „
For the formation of the antheridium	30 to 45 min.
From the complete formation of the antheri- dium to the commencement of oosphere differentiation	2 $\frac{1}{2}$ hrs.
From the commencement of oosphere differ- entiation to the first noticeable move- ment of protoplasm in the fertilization- tube (act of fertilization?)	2 „
From the act of fertilization to the rounding off and definite limitation of the oospore	1 „ (?)
For the maturation of the oospore	24 „

Ripe oospores may, as a matter of fact, be obtained in abundance on the fourth day in suitable cultures. Three- and four-day cultures would then be generally suitable for the preparation of serial stained sections, and were indeed most frequently used.

Germination of the oospores. The oospores germinate as soon as ripe, or after a period of rest extending to seven months. The process of germination resolves itself into two stages, which are in my experience generally separated by a period of rest. In the first stage the *oospore becomes a*

conidium, in the second this conidium develops one or more germ-tubes. To provide suitable material for sectioning, it was necessary that large numbers of oospores should be made to germinate together. After many futile attempts, in which the effects of variations in temperature and culture media were tried, success was achieved as follows:—

A mass of oospores was allowed to stand for some days in distilled water. These were then placed in running water and examined from time to time. Large numbers of oospores germinated. Upon examining such oospores it was found that (1) the thick inner layer of the wall of the oospore dissolved, (2) the fatty globule disappeared together with the lateral nucleus, and (3) the protoplasm increased in amount—no doubt at the expense of the reserve materials, and became more coarsely granular. The oospore in this changed condition resembles a conidium so closely that its real character is recognizable only by its position inside the wall of the oogonium. In running water no further development takes place as a rule, but if such material be transferred to cabbage-water the germination is completed by the outgrowth of one or more germ-tubes, such as are produced by ordinary conidia.

Once again it is to be noted that under no circumstances is there the slightest tendency to the formation of zoospores. Whatever the age of the oospores, the nutrient medium in which they are placed, or the temperature to which they are subjected, tube germination takes place or no germination at all.

However the conditions which regulate the germination of the oospores still require further investigation, as the following considerations will show. The method outlined above does not *always* succeed. The reasons for the failures have to be determined. Further, when the transference of the half-germinated oospores to the cabbage-water is made it is found that only a very small percentage (5 to 10) succeed in developing germ-tubes. The remainder burst, liberate a portion of their contents under considerable pressure into the

cavity of the oogonium, and then perish. A profile view of the bursting has not yet been obtained, although about six specimens have behaved in this way under direct observation. For these reasons figures illustrating the germination of the oospores are for the present withheld. The actual outgrowth of the germ-tubes has been traced under the microscope in about thirty cases, occurring in two sets of cultures in two successive years.

Interesting differences presented by the three types of cultures. The foregoing remarks refer almost exclusively to observations carried out on moist-chamber cultures. These are apt to be abnormal for the reasons already given, and it was thought wise to grow the plant in Petri dishes in abundance of water to check the results obtained by direct observations of small cultures under the microscope. In these cultures dead house-flies, which had been kept in an air-dry condition for twelve months, and bits of cabbage leaves were generally used to furnish the requisite nutrient material. Both kinds of food were sterilized by steaming for an hour on each of three successive days. Having set up at least sixteen series of Petri-dish cultures, each with four or more examples, one can safely make the following generalizations. The cultures on cabbage leaves were more luxuriant than those on the flies, and they normally produced oospores only. The house-fly cultures, on the other hand, produced with few exceptions conidia only. In cabbage-leaf cultures conidia might occasionally be seen, but always in relatively small quantity, and similarly in house-fly cultures a few oogonia might be found. By using these two types of cultures for imbedding, it was obviously easy to avoid confusing sections of oogonia and conidia. Such an aid was, however, found to be quite unnecessary, for these organs are much more readily distinguished in the stained than in the fresh condition.

It has already been said that pure cultures were made in August, 1900, on potatoes. These cultures were remarkable not only for the luxuriant development of an aërial mycelium, but for the fact that they remained sterile for weeks. At

first indeed it was thought that they would prove permanently sterile. However, a culture six weeks old acquired patches of golden yellow colour, and an investigation of the reason for the change led to the discovery of both kinds of reproductive organs—conidia and ripe oospores, chiefly the former. Many of the cultures perish through drought before producing spores. We have here no doubt an interesting example of the effect of abundant food material, as already noted for example by Klebs ('99) for *Saprolegnia mixta* and by Goebel ('00) in the case of the protonema of *Bryum pseudotriquetrum*. The last culture set up was the thirty-first of a series, each of which was obtained by inoculation with a sterile fragment of its predecessor. The mycelium in this last culture is a direct descendant (by vegetative propagation) of that first obtained in August nearly five months ago, and still shows its tendency to remain sterile, while the abundant supply of nutriment remains unexhausted.

These interesting differences in the behaviour of the plant under different external conditions require further investigation. I hope to be able, at some future date, to give a more satisfactory account of them in a paper on the physiology of the species.

Dissemination of the spores. The conidia and oospores (or oogonia) are set free by the rotting away of the old exhausted mycelium. In pure cultures they may remain attached apparently for an indefinite period. In nature no doubt the spores are liberated and disseminated by the disintegration of the rotten substratum upon and in which they are found. No experiments have yet been made on their powers of resisting drought. The spores, however, do not appear to be adapted for dissemination by the wind. Potatoes have been purposely left exposed in the laboratory in places where enormous numbers of spores had been produced, yet no spontaneous infection took place. The mode of dissemination indicated is, however, effective enough for a plant which vegetates in the surface soil on rotting vegetable and animal matter.

Relation of growth to temperature. The cultures were carried on at first during the summer months, and growth and development was, as has been said, very rapid. After a lapse of two months fresh cultures were started—in November, 1899—and these made very poor growth, and the reproductive organs appeared only after the lapse of a considerable time—a week or more. A few experiments sufficed to prove that this was entirely the result of the low temperature. Potato cultures do not produce an appreciable aërial growth at room temperature during our winter, so that in the winter months the cultures have to be kept growing in the incubator. Growth is very good, at its optimum probably, at a temperature of 25°C . The incubator is maintained at 24°C . to 26°C . Growth is stopped at about 35°C . The optimum, minimum, and maximum temperatures have yet to be accurately ascertained, but we already know enough to realize that the plant is adapted for a summer rather than a winter life in our climate. This is the more remarkable, as our wet, mild winter would appear to furnish it with a very suitable environment.

Experiments to determine its capacity to vegetate as a parasite. From the outset the plant appeared to be a pure saprophyte, but as it was of importance for taxonomic purposes to determine the point accurately, two sets of experiments were instituted. *In the first*, cultures of cress-seedlings were raised in sterilized soil in a damp atmosphere under bell jars. These were infected with healthy cultures of the Fungus, chiefly by laying the cover-glass cultures on or in the soil in contact with the plants. These cultures of the Fungus were not absolutely pure, and free watering was resorted to, as at that time the power of the mycelium to grow in the air was not known. No single case of 'rotting off' occurred in the six experiments instituted. Indeed the cress developed quite luxuriantly under these conditions. *In the second*, cress-seedlings were uprooted and laid in water in sterilized Petri dishes and cultures of the Fungus added. These cultures were to a considerable extent under microscopic control. No infection and little growth took place. The cress-seedlings

remained fresh for days. It must be remembered that if boiled cress-seedlings are used the material is attacked at once, and vigorous growth is the result. The species is clearly a true saprophyte.

OBSERVATIONS ON SERIAL SECTIONS.

Preparation of the material. At first Petri-dish cultures, three, four, or more days old, were taken and examined *in situ* under the microscope. If in suitable condition they were fixed in Hermann's fluid, while still in the dish and under microscopic control. They were then passed into paraffin through the medium of xylol, and the material was finally sectioned 5μ thick and stained with gentian violet. A complete series of preparations was found, illustrating all stages in the cytology, and then it became evident that the method was not an absolutely satisfactory one. The nuclei could not easily be followed at the stage immediately succeeding fertilization, as had been the case indeed in previous years with *Saprolegnia* and *Achlya*, and in addition there was a difficulty in following the details in the maturation of the oospores. The preparations used to furnish the illustrations were with four exceptions made as follows:—

Absolutely pure cultures were grown in Petri dishes and fixed under microscopic control by immersion in Flemming's stronger solution for periods of five hours or more. The blackening was removed by hydrogen peroxide and the dehydration carried out with great caution. No collapse took place. Here the microscopic control came to an end. The material was transferred to paraffin through the medium of xylol. The alcohol was gradually displaced by xylol and the xylol by paraffin. Notwithstanding these precautions, shrinking and some amount of collapse took place at this stage, although curiously enough chromacetic acid material passed through the imbedding oven at the same time under precisely similar conditions showed no shrinking of any kind. Sections were cut of 5μ thickness and stained with gentian violet.

For purposes of comparison other methods of fixing and staining were used. Mercuric chloride solutions, platinum chloride solutions, and acetic alcohol were used for fixing, in addition to those already mentioned. The best results were obtained with Flemming's solution, chromacetic acid, and Hermann's solution, and of these the first two were somewhat better on the whole than the third. Flemming's solution preserved the details of the protoplasm better than the chromacetic acid, and the nuclei could be followed at all stages with almost equal facility. The stains chiefly used were gentian violet, haematoxylin, and safranin. Gentian violet gave excellent results. Safranin proved useless although much time was expended over it. With gentian violet or iron-alum haematoxylin it is possible to decolourize the cytoplasm and yet leave the whole nucleus either deep violet or black. The counting of the nuclei is very easy in such cases. By further differentiation the detailed structure of the nucleus is disclosed. With safranin diffuse staining was alone possible. After many experiments with two kinds of safranin (Grübler's), using the stain alone and in combination, no single section was found with good differentiation.

It is probable that equally good results would have been obtained with the material fixed in chromacetic acid, but time was not available for the detailed study of a third series of sections. It is noteworthy that although Flemming's solution kills the protoplasm instantly, and blackening is observable in a minute or two, very poor staining results were obtained when the time of action was limited to an hour. As Flemming has pointed out the special action of the chromic acid is a slow one.

Of the two series of sections fully examined, one was of material obtained from impure cultures in which Bacteria were present, the other was of material from absolutely pure and very fine cultures. These series were fixed and stained in different ways, but the results were always concordant. The pure cultures were of advantage, however, with respect to one point, as will be seen later. The illustrations, with

four exceptions, as already stated, are based upon preparations made from the pure cultures, fixed with Flemming's stronger solution for five or more hours, and stained with gentian violet.

The sexual organs and oospores. Figs. 6 to 45 represent consecutive stages in the development of the sexual organs, in fertilization, and in the maturation and germination of the oospores. The sequence may not be absolutely correct, but such errors as may unavoidably have crept in must be of small moment. Comparisons should be made with the corresponding figures representing the condition in the living material. Relative age may frequently be determined fairly well by the size of the organ, the distribution of the contents, and the thickness of the cell-wall.

At first it was intended to make careful countings of the nuclei in the different stages. This idea was soon abandoned, for even with sections 5μ thick the number of available sections in a series was generally but three, and so short a series is difficult to follow accurately because of the rapid change in the appearance of the sections. Indeed one has not only to find suitable sections, but sections so placed that there is no danger of passing from one oogonium to another. Moreover it was found that the median section of three was quite sufficient to give one a clear idea of the condition of the remaining two, and so of the whole. It is necessary perhaps to add that, nevertheless, a series of sections was generally examined before a median one was adopted for detailed study. The reader who has the drawings of the median sections before him has therefore a full opportunity of criticizing the views founded upon their examination. Had drawings of the adjacent sections been added in each case no additional feature of importance would be noticeable.

Fig. 6 represents a half-grown oogonium. The nuclei are small. The number is in this case exceptionally large, but the remaining two sections only contained four additional nuclei. The number of nuclei in a young oogonium is very variable, ranging from twelve to twenty-five or thirty, and the

distribution is by no means uniform. The oogonium has no central vacuole at any stage in its development.

In Fig. 7 the oogonium is completely formed, its basal wall being already present. The protoplasm is coarsely reticulate. The nuclei are relatively large and have arranged themselves at the periphery of a hollow sphere. This increase in size of the nuclei is in preparation for an indirect division. The arrangement of the nuclei in a ring prefigures the division of the contents of the oogonium into oosphere and periplasm. In another oogonium in the same stage of development, which had a diameter of 18μ , the nuclei were counted in the three sections. The numbers were ten, three, and one, making a total of fourteen.

In Fig. 8 the antheridium is shown in contact with the lateral wall of the oogonium. A 'receptive spot' has been formed by the oogonial plasm, somewhat similar to, although perhaps not so highly differentiated as those described by Wager ('96, '00) in *Cystopus* and *Peronospora*, and Stevens ('99) and Davis ('00) in *Albugo* (*Cystopus*) *Bliti* and *Albugo* (*Cystopus*) *candida*. A small nucleolus is visible in the oogonial nuclei at this stage.

In Fig. 9 the formation of the egg has commenced, the fertilization-tube is in contact with the dense protoplasm, and the ring-like grouping of the nuclei is very noticeable. The nuclei are in metaphase, the spindles being plainly seen in the oogonium and antheridium. One oogonial nucleus is inside the ring formed by the others. The number of separate chromosomes visible in a polar view of one of the dividing nuclei is 8.

In Fig. 10 the nuclei are in anaphase, and the connecting threads of the achromatic figure are clearly visible. The egg is outlined, and one nucleus only is dividing inside it; the rest are in contact with its outer surface.

In Fig. 11 the fertilization-tube has penetrated the periplasm quite far enough for its apex to be invisible, except with the aid of sections. Here two nuclei are still in the egg, and nuclear division is not so far advanced as in Fig. 9, although

in that case no differentiation of the egg had taken place. It is obvious that the development of the egg, growth of the fertilization-tube, and nuclear division are, to some extent at least, independent processes.

In Fig. 12 the egg is much more clearly outlined, and contains one nucleus probably in metaphase. In Fig. 13 the fertilization-tube is in contact with the egg, which however is by no means ready for fertilization. The periplasmic nuclei have divided and are obviously smaller and more numerous. The egg contains four nuclei, the result of a recent division, one division indeed being not quite complete. The peripherally placed nuclei no doubt pass into the periplasm. Beautiful spindles are seen in the antheridium, showing that the nuclear divisions in the male and female organs are not quite synchronous.

In Fig. 14 the egg is relatively large, and its protoplasm is much less dense. The periplasm is thinner, but its nuclei show no sign of degeneration. The egg contains two nuclei, the one central and the other peripheral. In Fig. 15 the centre of the egg is occupied with a mass of protoplasm which stains more deeply than the remaining parts. This corresponds to the 'coenocentrum' of Stevens ('99). The detailed discussion of Swingle's ('98) view that it is an *organoid* of the cell, whatever that may exactly mean, would scarcely be a profitable one. It may be compared to a whirlpool in a river, for it has a form, though an inconstant one, and probably no real material existence. It is no doubt, as Stevens appears to have recognized, an expression of the forces acting at the centre of the egg rather than of the matter present there. The receptive spot is probably of a similar nature. It is convenient for purposes of description at all events to give such recognizable objects distinctive names. Although the term coenocentrum is not free from objection, it is adopted provisionally here as a useful descriptive term. The boundary between egg and periplasm is very sharply defined in this figure. The end of the fertilization-tube has penetrated into the egg.

although the nuclei in the antheridium are still undergoing division.

In Figs. 16 *a* and 16 *b*, representing adjacent sections of the same oogonium, the 'coenocentrum' is present and the egg fully differentiated. The periplasmic nuclei are relatively large, and the fertilization-tube just reaches to the egg. Sections like these when compared with those represented in the two preceding figures arouse the suspicion that there may be a double division of the nuclei. The weight of the evidence, based upon countings of the nuclei, is against a second division. Such differences as are presented here we must regard, at any rate provisionally, as due to simple variation. In Fig. 17 the egg is uninucleate but destitute of a 'coenocentrum,' yet the fertilization-tube has penetrated a considerable distance. The nucleus in the antheridium is in metaphase. In Fig. 18 the fertilization-tube is almost in contact with the female nucleus. Nuclear division in the antheridium is almost complete. One pair of daughter-nuclei are seen in the last stages of division.

In Fig. 19 we have before us apparently the very act of fertilization. The egg has now separated from the periplasm, and the 'coenocentrum' is very obvious. The fertilization-tube can be traced almost as far as the 'coenocentrum' and up to the posterior end of an elongated male nucleus. The wall of the fertilization-tube may be traced as a transparent membrane of measurable thickness through the periplasm up to the margin of the egg, but its continuation in the egg itself is no longer recognizable as a definite membrane. It must be remembered that such sections as these, stained to show the structure of the cytoplasm and nuclei and mounted in balsam, are not suitable for the study of the cell-membrane. The nature of the boundary line between the substance of the egg and the fertilization-tube which lies within it, must therefore, for the present at least, be left undecided. It is, however, abundantly clear from the preceding figures that the general view as to fertilization in *Pythium*, founded on De Bary's observations on living material, is for this species

certainly, and probably for the others, an erroneous one. There is no separation of the antheridial contents into periplasm and gonoplasm. In the oogonium, the periplasm covers the egg with a dense mantle at the period of fertilization, and makes it impossible to follow the actual process in the living material. The passage of protoplasm down the fertilization-tube has been assumed to be a proof that the act of fertilization was going on, an assumption entirely unwarranted now that we know the central mass is not an egg, but an egg covered with a thick dense mantle of periplasm. Further examination of Fig. 19 reveals the interesting fact that degeneration of the periplasmic and supernumerary antheridial nuclei has commenced. The degeneration is characterized by the assumption of greater density and great irregularity of form. The vesicular nuclei, in fact, appear to collapse.

The next figure is of a section of considerable interest. It and one other, unfortunately insufficiently noted and passed over before its importance was recognized, were the only sections found in this condition. Of other stages, with the exception of that in Fig. 19, many examples have been seen. The egg in Fig. 20 is only partially separated from the periplasm. It is uninucleate, presumably ready for fertilization, and is deeply indented by the inpushing of the fertilization-tube. That there is a definite boundary to the fertilization-tube in this case, is shown by the slight plasmolysis or shrinking which has taken place. The other section, incautiously regarded as anomalous, and lost after making a rough sketch of it, showed the egg quite free from the periplasm, but deeply indented on one side by a swollen fertilization-tube. There was the same shrinking as in the previous case, but the egg was binucleate. One nucleus, no doubt the female nucleus, occupied the centre of the egg; the other was just within the periphery of the egg at the bottom of the indentation, and immediately opposite the fertilization-tube, which however appeared to have a closed end. The inference drawn from these observations is that the fertilization-tube parts with a nucleus at its tip, just as a tube filled with a viscous

liquid may be easily made to part with a single drop from one extremity. This nucleus fuses with the egg at the moment of its liberation. The egg then rounds itself off, pushing out the thin-walled portion of the fertilization-tube to the periphery and so into the periplasm. A portion of the antheridial contents probably passes into the periplasm, the rest remains behind in the antheridium. The whole process probably depends upon, and can be explained in terms of, chemiotaxis, osmotic pressure, and surface tension.

Figs. 21 to 27 show important stages in the maturation of the oospore. In each case there are two nuclei. Hundreds of oospores have been examined in this condition, and all were binucleate. If a section proved to be uninucleate, as sometimes happened, one could with certainty predict that the remaining nucleus would be found in the adjacent section. It is noteworthy that the cell-membrane of the young oospore remains very thin and delicate throughout the period represented in these sections, which, however, is no doubt a short one. Nuclei in a degenerate condition may be found in the antheridium, as shown in Fig. 22, after the membrane of the spore is fully formed. The order of succession in this series is marked by one character only, that of the gradual disappearance of the periplasm. The collapsed nuclei degenerate rapidly. They are apt to form irregular figures, as shown in Figs. 22 and 23, and vanish altogether before the last traces of protoplasm have been dissolved.

The significance of these observations is perfectly clear. The whole of the periplasm is digested and absorbed by the young oospore, an actual increase in size of the latter being noticeable during the process. The explanation I ventured to give of the fate of the supernumerary nuclei in *Saprolegnia* and *Achlya* finds confirmation here. Working with pure cultures proved of advantage in this case. Under such conditions there could be no question of some outside agents like Bacteria acting as ferments and absorbing the products. The plant itself was the only possible agent concerned, and if we consider how rapidly the result is effected, and the growth

of the oospore which simultaneously takes place, we may with safety conclude that the whole process of digestion and absorption is the work of the young oospores themselves. It is well to note that no appreciable thickening of the oospore-membrane takes place until the last traces of periplasm have disappeared. The first stage in the maturation of the oospores is marked then by the digestion and absorption of the periplasm by the growing oospores.

The second stage, represented in Figs. 28 to 32, is marked by three distinct processes of differentiation, viz. the thickening of the spore membrane, the fusion of the male and female nuclei, and the development of the reserve globule. The primary membrane of the oospore remains thin and appears to become slightly cuticularized, staining deeply with gentian violet. New layers are, however, added on the inside at the expense of the protoplasm, until a very considerable thickness is reached. Whether we are justified in speaking of an exospore and endospore in this case is doubtful, for as we shall see, the very thick inner wall is essentially a mass of reserve material. The two nuclei approach each other—as indeed they do at an earlier stage, as may be seen by comparison of Figs. 25 and 28—but the actual fusion is delayed for a considerable period. Apparently ripe oospores are sometimes found to contain two closely approximated nuclei, as shown in Fig. 32, but this is very exceptional. The ripe oospores are uninucleate, and uninucleate oospores may be found in cultures four days old. The actual fusion is not difficult to trace, as the nuclei at this stage are in the resting condition and fairly conspicuous objects. Three stages in fusion are represented in Figs. 29, 30, and 31. In the ripe oospores there is constantly to be found a globular mass which in the main gives the proteid reactions. This mass may have one large cavity in its interior, when its form becomes that of a hollow sphere; or it may have numerous small cavities, in which case it possesses a spongy character. The mass corresponds to the reserve globule seen in the living material. It has generally been considered to be of

a fatty nature. More probably it represents a complex aggregate of oils and proteids. Its thorough chemical investigation is much to be desired. Its origin has not been traced.

Figs. 33 to 40 illustrate sections of germinating oospores drawn specially to show the behaviour of the nucleus at this period, sections showing the reserve globule being consequently excluded. In Fig. 33 the large nucleus is at rest and has a conspicuous nucleolus. The reserve globule is shown in this case for the sake of comparison. In Fig. 34 the nucleus is in the spirem condition and the nucleolus still visible. In Fig. 35 the nucleus is in metaphase and the chromosomes may be approximately estimated. At one end of the spindle six can be counted. Such nuclei are practically indistinguishable from those seen undergoing karyokinesis in the sexual organs. It is not possible to make an accurate estimation of the chromosomes in each case, but the appearances all point to the numbers being approximately equal. In Fig. 36 there are two nuclei undergoing division, and at this stage the inner wall of the oosphere is much thinner than before. The protoplasm obviously grows at the expense of the inner wall. In Figs. 37 and 38 we have further solution of the wall and division of the nuclei.

In Figs. 39 and 40 the wall is thin and delicate, enclosing granular reticulate and vacuolated protoplasm and numerous nuclei. Six to eight nuclei may be counted in a single section. Additional nuclei would of course be found in the sections adjacent to those represented in Figs. 39 and 40, but not in those adjacent to the ones represented in Figs. 33, 34, 35, and 36. Such sections of oospores as are shown in Figs. 39 and 40 could not be distinguished from those of conidia but for the presence of the oogonial wall. It is especially noteworthy that at this stage, as represented in Fig. 40, some of the nuclei appear to be double. Careful observations show that the double condition depends upon the presence of two central chromatic masses (not nucleoli). The most obvious explanation of this is that the nuclei are undergoing more or less

direct *divisions* or *fusions*. Another explanation might be found in the supposition that the appearances were caused by two nuclei coming into contact with one another. We have to note, however, that the nuclear membrane is distinctly traceable as a definite line, sometimes dipping inwards so as to cause partial constriction, but never separating the two chromatic masses from one another. Further, the number of nuclei remains fairly constant when these abnormal nuclei are present. There is neither a diminution nor increase in their number, and indeed they appear in the reproductive organs only when these are at rest. At present it is therefore impossible to do more than regard them as nuclei with anomalous structure. The future may be able to throw light on their genesis and fate.

Figs. 41 to 45 illustrate sections of germinating oospores designed to show the fate of the reserve globule. Incidentally they show the changes in the wall and the nuclei at the same time. It is clear that the reserve globule becomes more spongy, breaks up into separate masses and gradually undergoes digestion, absorption, and assimilation. The last traces of this reserve material may be seen in the form of deeply stained granules in Figs. 44 and 45.

We have still to consider the structure of the mycelium and conidia as traceable in sections, and the phenomena associated with the germination of the latter. Fig. 46 is that of a section of a young conidium obtained from a four-day culture, Fig. 47 that of a conidium fixed when about one month old. The first was fixed in Hermann's solution, the second by immersion in Flemming's stronger solution for one hour. Observation of the living material had led me to expect differences in the number of the nuclei. There were, however, no differences of importance observable. The difference in the number of nuclei observed in the sections comes well within the limit of variation. One could in fact obtain scores of sections in one slide with the numbers in the opposite proportion. The older conidia are generally vacuolated, and the unequal distribution of the vacuoles leads to an unequal

distribution of the nuclei. The section adjacent to that represented in Fig. 47 was conspicuous for its vacuoles and the small number of nuclei. The following countings may be of interest. One section of a conidium 12μ in diameter had three nuclei, five sections each 14μ in diameter showed respectively 8, 7, 8, 6, and 5 nuclei, and four sections each 16μ in diameter showed respectively 10, 6, 6, and 11.

Figs. 48 and 49 are drawn from material preserved in chromacetic acid. Fresh conidia were placed in cabbage-solution for about an hour and then fixed. The nuclei are very distinct, but the finer details are not so sharp as in material fixed with Flemming's or Hermann's solutions. The beautiful reticulate structure of the cytoplasm is likewise absent. The plane of section in the case shown in Fig. 49 was parallel to the direction of one of the germ-tubes. One spindle is seen in these figures quite comparable to those present at other stages, and three cases of 'double' nuclei. One is obviously justified in describing the first stage in the germination of the oospores as the conversion of the oospore into a conidium. One can easily understand, now that the cytological details are known, the peculiar behaviour of the oospores in germination. There appears to be no necessity for tracing the development of the germ-tube in the germinating oospore, for the behaviour must correspond exactly to that described and figured in the case of the conidia. The structure of the hyphae has been frequently observed, and corresponds to that shown in the germ-tube of Fig. 49. We have then before us a clear picture of the cytological details at all stages of the life history. Certain gaps in our knowledge, indeed, remain to be filled up, but these are of minor importance.

It may be of advantage to bring together the observations made on the structure of the nucleus. The resting nucleus is vesicular with a distinct nuclear membrane. In the centre is a chromatic mass, and traversing the 'nuclear sap' between this and the nuclear membrane is a very delicate network of 'linin' threads with very little chromatin attached to them.

When karyokinesis begins the chromatic mass gradually breaks up, the chromatin passes out on to the linin threads, and a distinct nucleolus remains behind. As the spirem stage is reached, the network of linin bearing the chromatin granules becomes very evident. The nucleolus sometimes persists until the spindle has been fully formed, and even until the metaphase condition is reached. The nuclear membrane can be recognized until the nucleus is in metaphase, but at later stages it is not possible to trace it definitely. The achromatic figure appears to arise entirely within the nucleus, and of its threads those which serve to connect the daughter-chromosomes can be readily recognized in the last stages of division. The number of chromosomes is certainly as many as six, probably more. It is difficult to get definite results on this point. If the stain is too deep there is a danger of counting two adjacent chromosomes as one; if not deep enough the chromosomes are apt to be overlooked. The chromosomes are, in fact, so small that deep differential staining and the best objectives are necessary for their proper resolution. The nuclei, at all stages in the life history, agree in general appearance when seen in division, the impression being created that there is *no variation* in the number of the chromosomes.

OBSERVATIONS OF TAXONOMIC INTEREST.

At an early stage of the investigation it became apparent that the Fungus was either a species hitherto undescribed or described so inadequately as to be difficult of recognition. The knowledge which we have gained of its characters leaves no doubt as to the affinities of the species. It resembles *Pythium vexans* most closely, a species, as Fischer ('92) points out, incompletely investigated and inadequately described by De Bary ('81).

De Bary's figures of *P. vexans*, however, show conclusively that our species is a distinct one. In fact, if we are to unite it with *P. vexans* we must ascribe to De Bary a carelessness and want of insight altogether foreign to that brilliant and

indefatigable botanist, to whose labours we owe so much of our knowledge of the Peronosporaceae.

As the species stands at the end of a series indicating greater and greater adaptation to a terrestrial existence, as evidenced by the fact that it alone has lost all power of producing zoospores, I propose for it the name *Pythium ultimum*. The specific name will thus serve to call attention to its position in the genus. The species may be defined as follows:—

Pythium ultimum, n. sp.

Mycelium saprophytic on boiled potatoes, house-flies, cabbage leaves, and other vegetable and animal substrata, never parasitic; either aerial and then very luxuriant, and snow-white like cotton 'wool' (as in potato cultures), or aquatic and inconspicuous; extramatrical and intramatrical; hyphae very long (6 cm.) and slender, the diameter varying from 6.5μ to 1.7μ (average of sixteen measurements = 3.8μ), much branched, septate in old cultures, the septa separating the older empty parts of the hyphae from the younger.

Conidia chiefly terminal and spherical, with a diameter varying from 28μ and more to 12μ and less (average of 25 measurements = 20μ), but occasionally intercalary and barrel-shaped, with the dimensions varying from $27.8\mu \times 22.9\mu$ to $17\mu \times 14\mu$; set free by the decay of the supporting hyphae and germinating at once in nutrient solutions (in an hour if placed in cabbage-water) with the formation of one or more germ-tubes; remaining at rest in exhausted nutrient solutions or distilled water for so long a period as seven months without loss of germinating power, and showing no tendency to form zoospores either in distilled water or running tap-water; multinucleate.

Oogonia, terminal and spherical, very rarely intercalary, with a diameter ranging from 22.9μ to 19.6μ (average of 14 measurements = 20.6μ); smooth.

Antheridia, generally one to each oogonium, arising from the stalk of the oogonium immediately below its boundary

wall, curved; sometimes especially in luxuriant cultures, two to an oogonium, and then often of diclinous origin and frequently straight.

Oospores, one in each oogonium, spherical, the diameter varying between $18.3\ \mu$ and $14.7\ \mu$ (average of fourteen measurements = $16.3\ \mu$) with a smooth thick two-layered wall of yellowish colour, enclosing finely granular cytoplasm, a central reserve globule and one lateral nucleus; germinating at once or after a period of rest extending to seven months and always by one or more germ-tubes.

Zoospores are never developed.

Fischer ('92) in Rabenhorst's Kryptogamen Flora places *Pythium* in the Peronosporaceae. He further divides the Peronosporaceae into the Planoblastae and Siphoblastae. Of course the genus *Pythium* as originally defined was planoblastic, but, as we have seen, *Pythium ultimum*, while agreeing with its fellows in other respects, is siphoblastic. This subdivision of the Peronosporaceae is not based on a sound principle, for even if we regard *Pythium ultimum* as an exception, similar remarks apply to the old genus, *Peronospora*. The subdivision proposed involves the separation of *like* from *like*.

Schröter ('93) in the Pflanzenfamilien places *Pythium* in a special group—the Pythiaceae—and this has much to recommend it, as *Pythium* differs very considerably from the other members of the Peronosporaceae. The Pythiaceae are, however, separated from the Peronosporaceae, and included with the Saprolegniaceae in a group, the Saprolegniineae. Schröter was probably greatly impressed with the similarity in the 'habit' and the 'mode of life' of certain species of *Pythium* and many members of the Saprolegniaceae. It must be remembered, however, that none of the Saprolegniaceae produces a periplasm, and this difference in character in itself is quite sufficient to justify us in preferring Fischer's classification. It may be added that I have found the cell-wall in Saprolegniaceae to consist invariably of typical cellulose. In *Pythium*

ultimum the cell-wall does not readily give the cellulose reaction with iodine and sulphuric acid. The reason for the difference has still to be accurately determined. The sum of the characters in *Pythium*, which is of course the best test of affinity, compels us to assign it a place in the Peronosporaceae. In this group it occupies, as is generally admitted, the lowest place. The adherence to the aquatic habit, the frequency of the saprophytic mode of life, the rudimentary type of oospore wall, and the crude nature of its attack on its host—if parasitic, destroying rather than enslaving—serve to distinguish it sharply from the more highly placed genera such as *Peronospora* and *Cystopus*. It would be interesting to have a more detailed knowledge of *Phytophthora*. In the present state of our knowledge it seems to be more closely related to *Pythium* than to the other two genera, and to occupy roughly an intermediate position.

GENERAL CONSIDERATIONS.

It had been hoped that incidentally this research would have thrown some light on that difficult and engrossing problem—the alternation of generations in plants. I ('99) have already indicated in the *Annals of Botany* how a study of the cytology of the Thallophytes may be expected to throw light on this question. It may be well to call to mind that plants are known to possess two kinds of gametes, those as in *Fucus*, where the nuclei possess half the usual number of chromosomes; and those, as in *Lilium*, where the nuclei possess the full number. When the reduction in the number of chromosomes takes place in oogenesis and spermatogenesis, there cannot well be an antithetic alternation of generations. However great the diversity in the different generations due to special adaptation to the environment, we never get further than polymorphism of the gametophyte. When antithetic alternation typically occurs the reduction is known to take place during the development of the spores. The fact that plants certainly present these two definite cases is not even yet properly appreciated.

To which of these two cases does our plant belong? If a second division occurs in the gametangia, accompanied by a reduction in the number of chromosomes, then it obviously belongs to the first. If no second division takes place, it does not necessarily belong to the second. There may be a third type, with the features of which we are not yet acquainted. It would have, *of necessity*, to go into the second group only if it could be shown that a process of reduction in the number of chromosomes takes place in the germinating or resting zygote, similar to that which is supposed to take place in the embryo-sac mother-cell of *Lilium*.

We can, unfortunately, come to no definite conclusion from the facts hitherto disclosed in this species. The probabilities are that the gametes have nuclei with the same number of chromosomes as those of the vegetative nuclei. We are thus brought face to face with the fact that an apparently unnecessary nuclear division takes place. What is the significance of this division? The view advanced first by Hartog in a similar case and provisionally adopted by others, that it is an ancestral character with no present physiological significance, does not appeal to me if only for the reason that it tends to check further investigation. Moreover, its general occurrence in coenocytic plants of widely different character, such as *Cystopus Bliti*, *Peronospora*, *Saprolegnia*, *Achlya*, and *Pythium*, suggests that its present importance is by no means inconsiderable. The case of *Pythium* suggests the validity of the explanation which has already been advanced by Strasburger ('97), that these divisions are essential stages in the differentiation of gametes. As a general view the theory, however, does not appear to be admissible, for, according to Lagerheim ('00) and Oltmanns ('95) there do not appear to be any preliminary divisions in *Monoblepharis* and *Vaucheria*. In the Peronosporaceae and Saprolegniaceae the case is different. In *Pythium* in particular the unpractised eye cannot distinguish a conidium from a young oogonium. It is demonstrable too, in sections, that by the time the young antheridium makes its appearance the oogonial nuclei are preparing to divide.

Provisionally, at any rate, Strasburger's view may be adopted, and efforts should be made to extend our knowledge of the cytology of the gametangia and gametes in the sexual Thallophytes. It is probable that gameto-nuclei are much more susceptible to degenerative changes than vegetative nuclei. If so, the advantage of *all* the nuclei retaining their powers of division, rather than *one* only, is clear. The daughter-nuclei are more readily digested and absorbed.

Three types of fertilization have been described as occurring in the Peronosporaceae, but now that Fisch's type (*Pythium*) falls to the ground, there remain but two—the normal one common to most plants, and that described so fully by Stevens for *Cystopus Bliti* and styled concisely by Wager ('00), 'multiple nuclear fusions in pairs.'

Davis has endeavoured to harmonize these widely divergent types by deriving the Oomycetes from Zygomycete-like ancestors. It is perfectly easy to construct a subjective phylogeny in this way. Ideas of this kind are, however, of value chiefly in proportion to the amount of objectivity which they represent. It is just as easy to construct a phylogeny without reference to the Zygomycetes, which, though to a great extent theoretical, has a considerable foundation of fact.

We have in *Monoblepharis* a Fungus in which all the reproductive organs are still so much alike (sporangium-like) that their homology is at once apparent. Had the oogonium a number of oospores like that of *Sphaeroplea*, the resemblances would be more striking still. *Saprolegnia* offers little advance on the condition seen in *Monoblepharis*. The oogonia and sporangia are very similar. The numerous oospheres differ from the spores in size, owing no doubt to the fact that in their formation nuclei have been absorbed and digested. The successful nuclei preside over an unusual amount of cytoplasm. The antheridia are however reduced. The fewer the eggs, the smaller should the antheridia be. The reduction of the antheridium obviously goes hand in hand with the increase in size and diminution in number of the oospheres. Zooidiogamy

has moreover been replaced by siphonogamy, rendering a further reduction possible. The siphonogamous type of fertilization is an advantageous because an economical one, even in the case of aquatic plants. It is interesting to note that *Saprolegnia* is nevertheless not quite perfectly adapted to its environment, as it still wastes a considerable number of male nuclei. The homologies in *Saprolegnia* are sufficiently clear.

How are we to understand the case of *Cystopus Bliti*? Have we any facts to help us, or are we entirely dependent upon our imaginative powers? The homologies seem clear enough until we take account of the karyology. Let us remember what happens so frequently in the case of the sporangia of *Pythium* and *Peronospora*—genera closely allied to *Cystopus*. In these two genera, the sporangia, adapting themselves to new environments, are converted into 'conidia.' In some species the conversion is complete, in others, still in progress. Its characteristic feature is that one large coenocytic multinucleate conidium is developed in place of a sporangium, with an output of a number of small uninucleate zoospores. Does not the oogonium of *Cystopus Bliti* and other *Peronosporaceae* present to us the corresponding metamorphosis in the female gametangium? I think it does, and we are thus able by extending the view to the antheridia of the *Saprolegniaceae* and *Peronosporaceae* to get a clear, if novel, picture of the real condition of affairs in these curious plants. The fertilization-tube growing out from an antheridium towards the egg behaves *exactly* like the germ-tube of a conidium growing towards the leg of a fly. There are still difficulties, however, which need further explanation. It is especially necessary to seek some clue to the origin and significance of the periplasm. We can easily imagine how, owing to inequalities in the numbers of female and male nuclei, the excess of female nuclei were pressed out to the periphery. Or it may have been the amount of protoplasm requisitioned by each nucleus that led to this result—one exemplified by the *Fucaceae* at the present day. One can further imagine undesirable 'energids' being pressed out

altogether and not simply their nuclei. In this way the periplasm probably arose. There is nothing very remarkable in the fact that the male and female nuclei in *Cystopus Bliti* fuse in pairs, for this propensity they have no doubt inherited from their algal ancestors. We have now only to go a step further and imagine the habit of the nuclei of monopolizing the cytoplasm of their neighbours (as already seen in *Saprolegnia*), still further intensified to see how the uninucleate egg might arise in such forms as *Cystopus candidus*, *Peronospora parasitica*, and *Pythium ultimum*. There are many missing links in this chain of evidence no doubt, but perhaps one gap may be filled up by considering the behaviour of the nuclei in the differentiation of the oosphere in *Pythium ultimum*. More than one nucleus frequently remains behind in the egg to undergo division there. There is in this, at any rate, a suggestion of an ancestral condition in which numerous nuclei were present in the oosphere.

It is now apparent that the uninucleate egg of *Pythium ultimum* and its allies, is the homologue of the multinucleate one of *Cystopus Bliti*—in the terminology of Stevens is a 'compound oosphere.' In view of this relation it is almost necessary for Stevens to withdraw or amend his terminology. It certainly seems rather absurd to describe the uninucleate oosphere of *Pythium* as compound.

It is obvious that the uninucleated egg might have been evolved in other ways, but this view gives us a provisional explanation of the periplasm which is not wholly theoretical, whilst limiting our speculations to the Oomycetes themselves. The cytology of the Zygomycetes is so obscure at present, as Davis has well said; and the forms mentioned by him, in his attempt to create a phylogeny for *Cystopus Bliti*, show such remote affinity to the Peronosporaceae, that I feel it would be a mistake at present to direct attention to the Zygomycetes with a view to harmonizing these two types of fertilization. There certainly appears, contrary to the view propounded by Davis, to be no difficulty in connecting *Cystopus Bliti*, through forms like *Cystopus candidus*, *Pythium*,

Saprolegnia, and *Monoblepharis*, with algal ancestors of the type of *Sphaeroplea*, *Vaucheria*, or *Oedogonium*.

It may not be superfluous to add that whatever view may be taken of fertilization—whether it is regarded as a means of rejuvenescence or as the source of an indefinite number of fortuitous variations—the two types present in the Peronosporaceae are of almost equal efficiency.

It is noteworthy that Harper ('00) has just published an account of the fertilization-processes in *Pyronema* which serves to confirm to a considerable extent the hitherto unique observations of Stevens, and even perhaps to strengthen De Bary's view of the affinity between the Ascomycetes and Peronosporaceae.

Harper ('00, p. 380), however, does not appear to have grasped the significance of the facts published by Stevens, for he points out that the multinucleate conidia of *Cystopus* are not called 'compound,' although from analogy with the multinucleate oosphere that might have been expected. There is a very curious confusion of ideas here, from which Stevens himself is not exempt, if it be true, as Harper states, that he makes use of the term 'compound antheridium.' I have not been able, however, to trace this term to Stevens. The homologues of the oospheres are the sperms and zoospores, not the antheridia and sporangia. The conidia of *Cystopus* are sporangia, having indeed a considerable output of zoospores. The sporangium in this case has retained its characteristic power of producing zoospores, but acquired in addition a special capacity for dissemination. The antheridium and oogonium have, however, developed along different lines. The sperms which pass into the oosphere of *Cystopus Bliti* might collectively be called the compound sperm, just as the more definitely differentiated oosphere is called the 'compound oosphere.' To put the matter more plainly still, if De Bary's view of a gonoplasm could be shown to be true, and the gonoplasm were multinucleate, the gonoplasm would be a compound sperm. Similarly the conidium of *Pythium* is, phylogenetically considered, a compound spore. Stevens'

terminology, even if an unsuitable one, may serve a good purpose if it aids us in rearranging our ideas concerning fertilization. No clear idea of this complicated question, though, will be gained while gametangia are confused with gametes and sporangia with spores.

SUMMARY.

The conidia of this Fungus were found in rotten cress-seedlings in July, 1899, and the species has been cultivated as a saprophyte on sterilized potatoes, house-flies and cabbage leaves, &c., up to the present time (January, 1901).

The species appears to be a pure saprophyte, all attempts to infect fresh cress-seedlings having failed.

Pure cultures were obtained by infecting sterilized potatoes with material obtained from fairly pure cultures on rhubarb leaves.

On potatoes, an aërial mycelium is freely developed, which remains sterile for weeks. If the culture is prevented from drying up, crops of conidia and oospores are ultimately obtained.

An aquatic mycelium is produced in cultures grown on house-flies and bits of cabbage leaves immersed in water. The reproductive organs make their appearance when the culture is two or three days old, and as a rule the house-fly cultures produce conidia, and the cabbage-leaf cultures oospores only.

The whole life-history has been carefully followed in moist-chamber cultures. The chief features, as determined by De Bary and others for the genus, have been verified; but *in addition* it is clear (*a*) that De Bary erred in including the greater part of the periplasm in the oosphere, and in describing a sharp differentiation of periplasm and gonoplasm in the antheridium; and (*b*) that the periplasm is digested and absorbed by the young oospore, which, in consequence, increases in size.

The conidia and oospores invariably produce germ-tubes

on germination. No zoospores have been observed. The species is consequently new, and ranks as the most highly developed of the genus. The generally accepted definition and affinities of the genus require considerable modification.

The conidia germinate at once in cabbage-water, but remain at rest in distilled water. The behaviour of the oospores remains to be further investigated, but it is certain that they will germinate as soon as ripe, or after a rest of seven months.

The mycelium, conidia, oogonia, and antheridia are multinucleate, the oosphere and the ripe oospore uninucleate, and the young oospore binucleate.

The nuclei multiply in the mycelium and sexual organs by indirect division. No nuclear divisions have been seen in the conidia except during germination. No nuclear fusions take place other than those of the male and female nuclei in fertilization. The number of chromosomes is considerable, certainly six or more.

The oogonium, as it is formed, receives twelve or more nuclei, the antheridium three or more. These invariably divide once, so that the number of nuclei, although already greatly in excess of the requirements of the organs, is doubled. The supernumerary nuclei in the oogonium pass into the periplasm, one only remains behind and occupies the centre of the egg. No similar differentiation takes place in the antheridium.

The fertilization-tube penetrates the wall of the oogonium, passes through the periplasm, and penetrates deeply into the egg. One male nucleus passes down the tube and enters the egg. The oosphere clothes itself with a delicate cell-wall and proceeds to digest and absorb the periplasm, increasing in size during the process.

The male and female nuclei do not fuse until a thick oospore-wall has been produced. As the oospore ripens a reserve globule is formed in the centre of the oospore and the fusion-nucleus is forced to one side. No epispore is developed.

In the germination of the oospore, the reserve globule (probably consisting of proteids and fats or oils) and the inner wall of the spore are decomposed and absorbed, the nucleus undergoes division, and a conidium-like condition is acquired.

UNIVERSITY COLLEGE, CARDIFF,
January, 1901.

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EXPLANATION OF THE FIGURES IN PLATES XV AND XVI.

Illustrating Dr. Trow's paper on *Pythium ultimum*, n. sp.

All the figures were drawn with the help of the camera lucida. Figs. 1-5 are representations of living material, the remaining ones are drawings of stained sections. Those of the first set are magnified about 550, of the second about 1250 diameters. All the sections were 5μ thick. Median sections, i. e. the middle ones of series, are shown with one exception, viz. Fig. 16.

Fig. 1. Structure and germination of an intercalary conidium.

Fig. 2. Structure and germination of a terminal conidium.

Fig. 3. Stages in the development of an oogonium and antheridium.

Fig. 4. Further stages in the development of an oogonium and antheridium. Fertilization.

Fig. 5. Stages in the maturation of an oospore.

Figs. 6-32. Approximately successive stages in the development of the gametangia, in fertilization, and in the maturation of the oospores. Full description in the text.

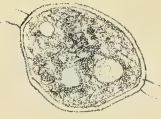
Figs. 33-40. Successive stages in the germination of the oospores, the fate of the reserve globule being neglected. The wall of the oogonium is represented in Figs. 38-40.

Figs. 41-45. Successive stages in the germination of the oospores, the fate of the reserve globule being followed. The wall of the oogonium is represented in Fig. 43-45.

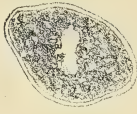
Fig. 46. Young conidium fixed in Hermann's solution.

Fig. 47. Month-old conidium fixed by immersion in Flemming's stronger solution for one hour, too short a time to secure good results.

Figs. 48 and 49. Conidia fixed in chromacetic acid while germinating. Chromic acid .7%, Acetic acid .3%.



1^a 9.58 a.m.



1^b 10.40 a.m.



1^c 10.48 a.m.



1^d 10.51 a.m.



9.20 a.m.



11.18 a.m.

1^f



1^e 10.57 a.m.



10.0 a.m.

2^a



2^b 11.2 a.m.



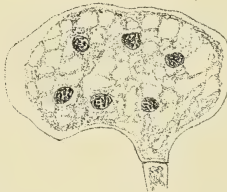
2^c 11.10 a.m.



2^d 11.21 a.m.



6.



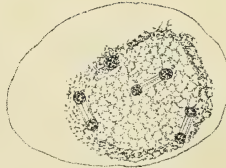
7.



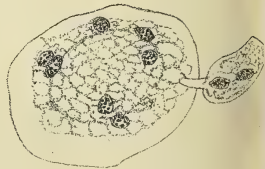
8.



9.



10.



11.

A.H.Trow, del.



11.50 a.m.

3^b



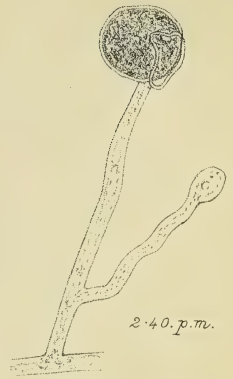
1.5 p.m.

3^c



2.10 p.m.

3^d



2.40 p.m.

3^e



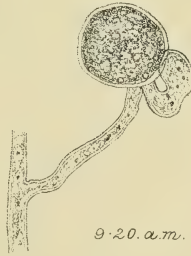
4^a

a.m.



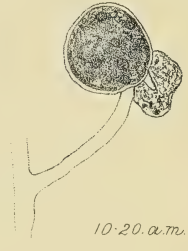
8.30 a.m.

4^b



9.20 a.m.

4^c



10.20 a.m.

4^d



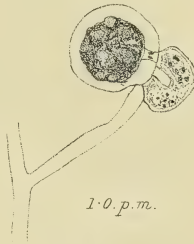
11.20 a.m.

4^e



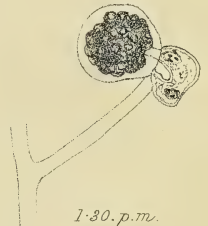
12.0 noon.

4^f



1.0 p.m.

4^g



1.30 p.m.

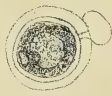
4^h



5^a 11.0 a.m.
30.8.99.



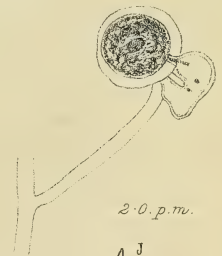
5^b 11.0 a.m.
31.8.99.



5^c 11.0 a.m.
1.9.99.

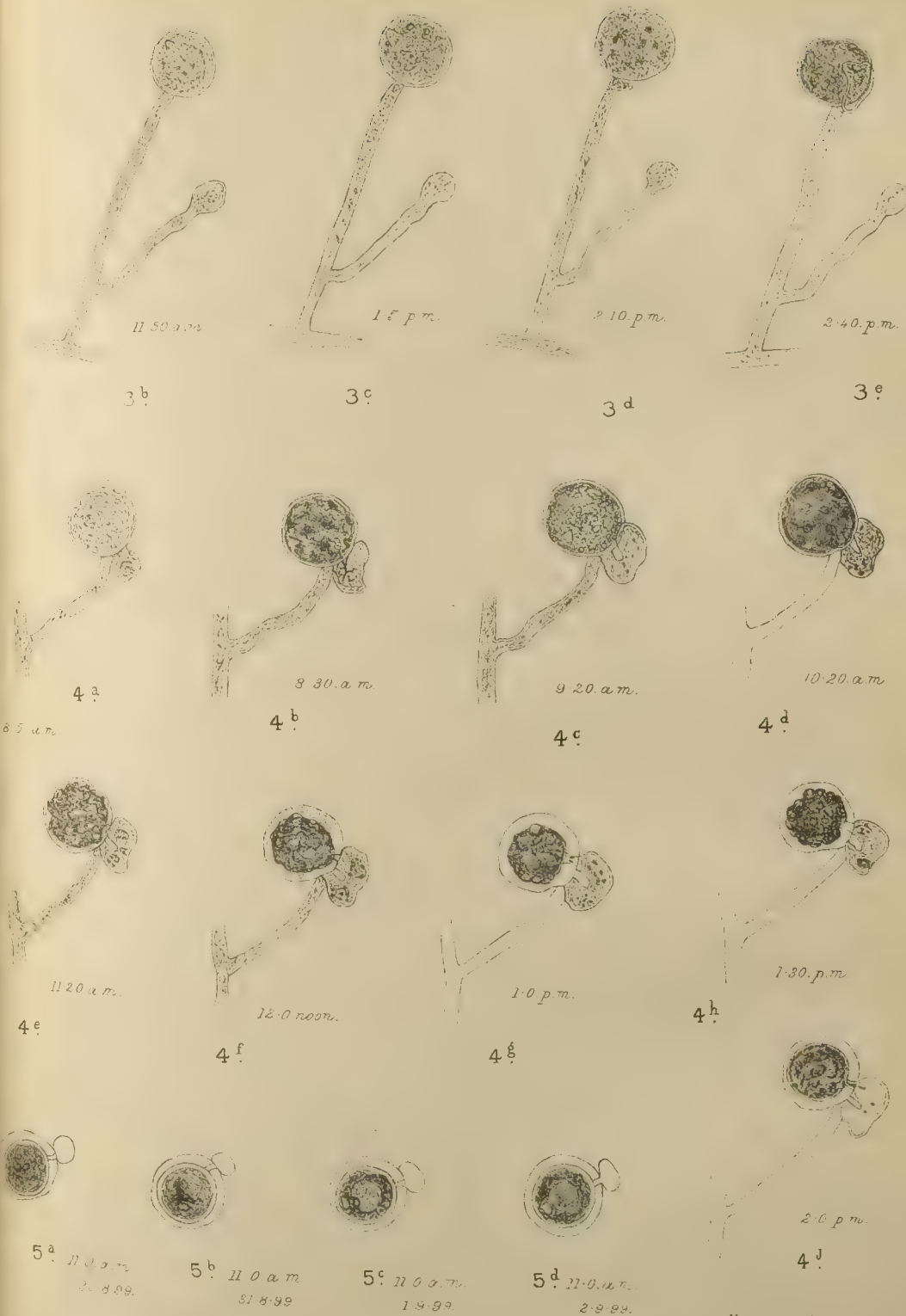
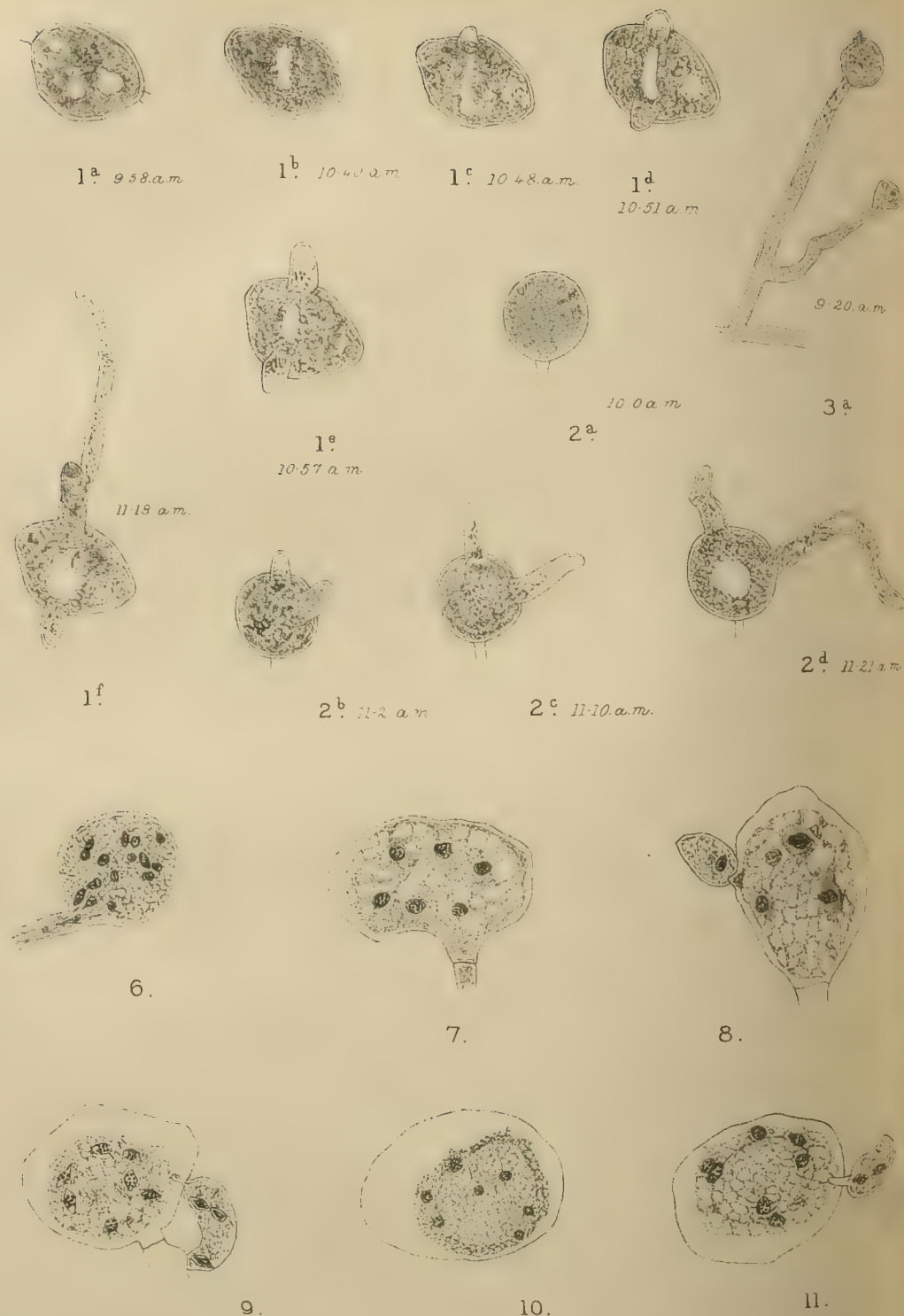


5^d 11.0 a.m.
2.9.99.



2.0 p.m.

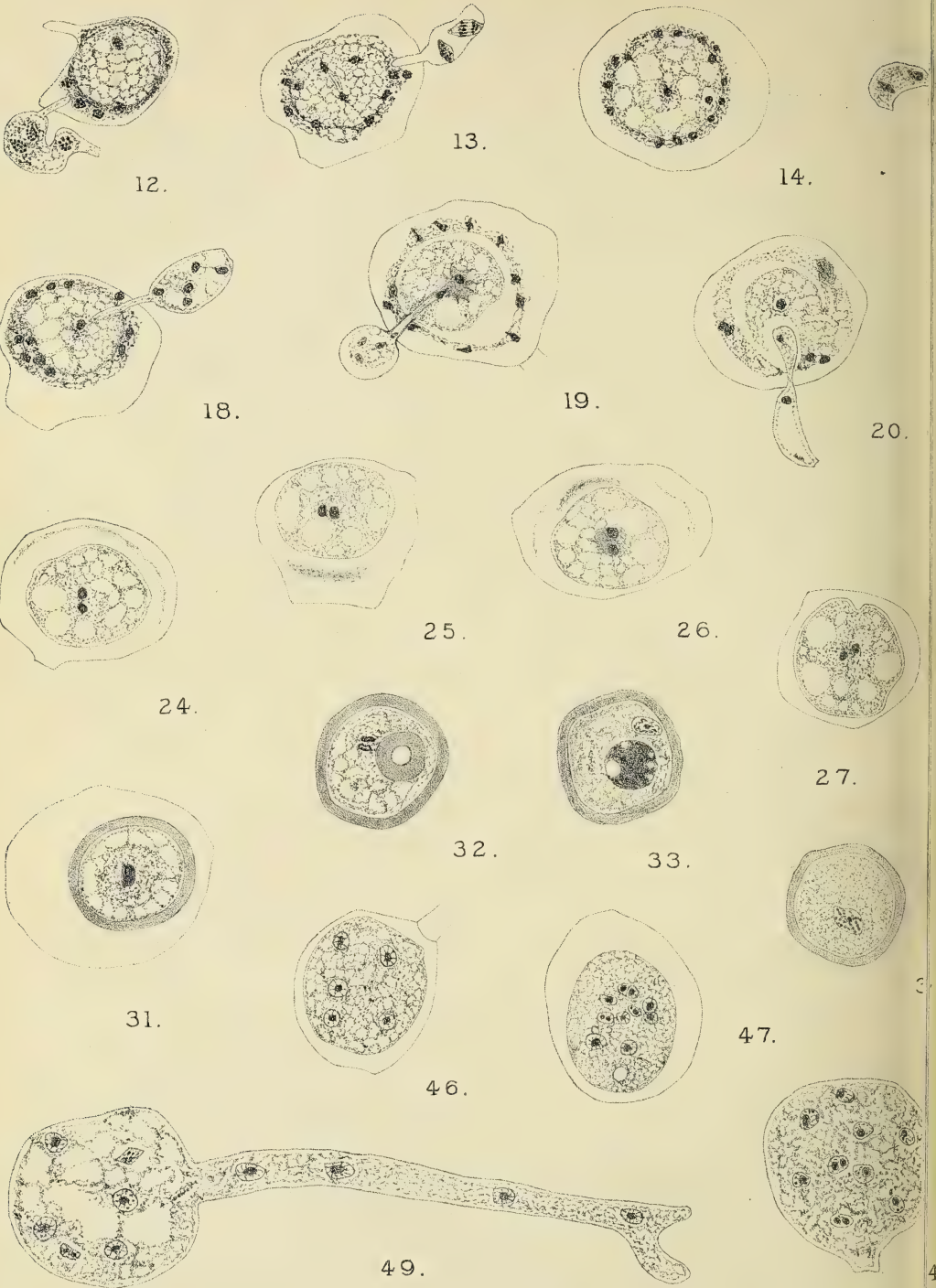
4^j



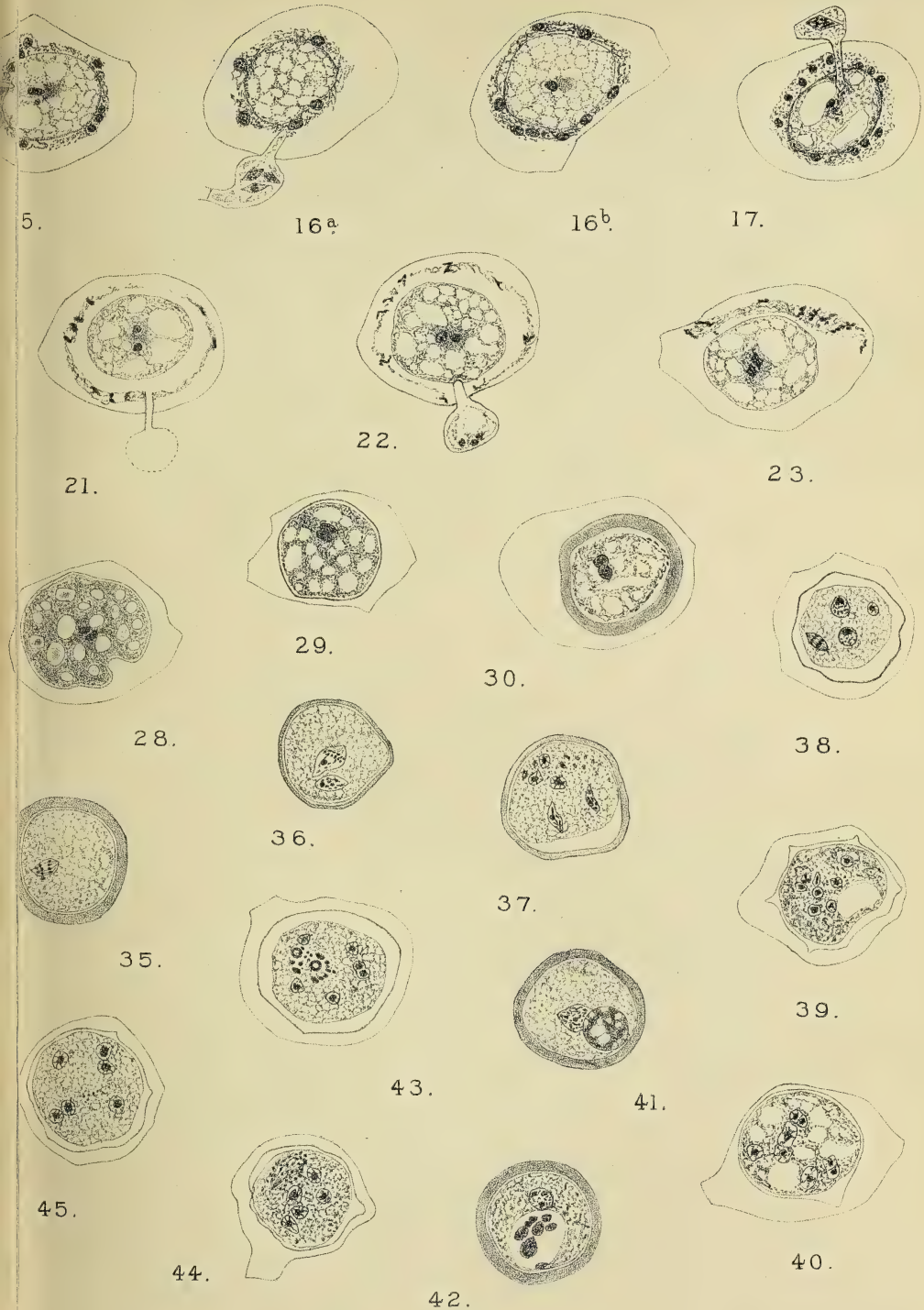
A.H. Trow, del.

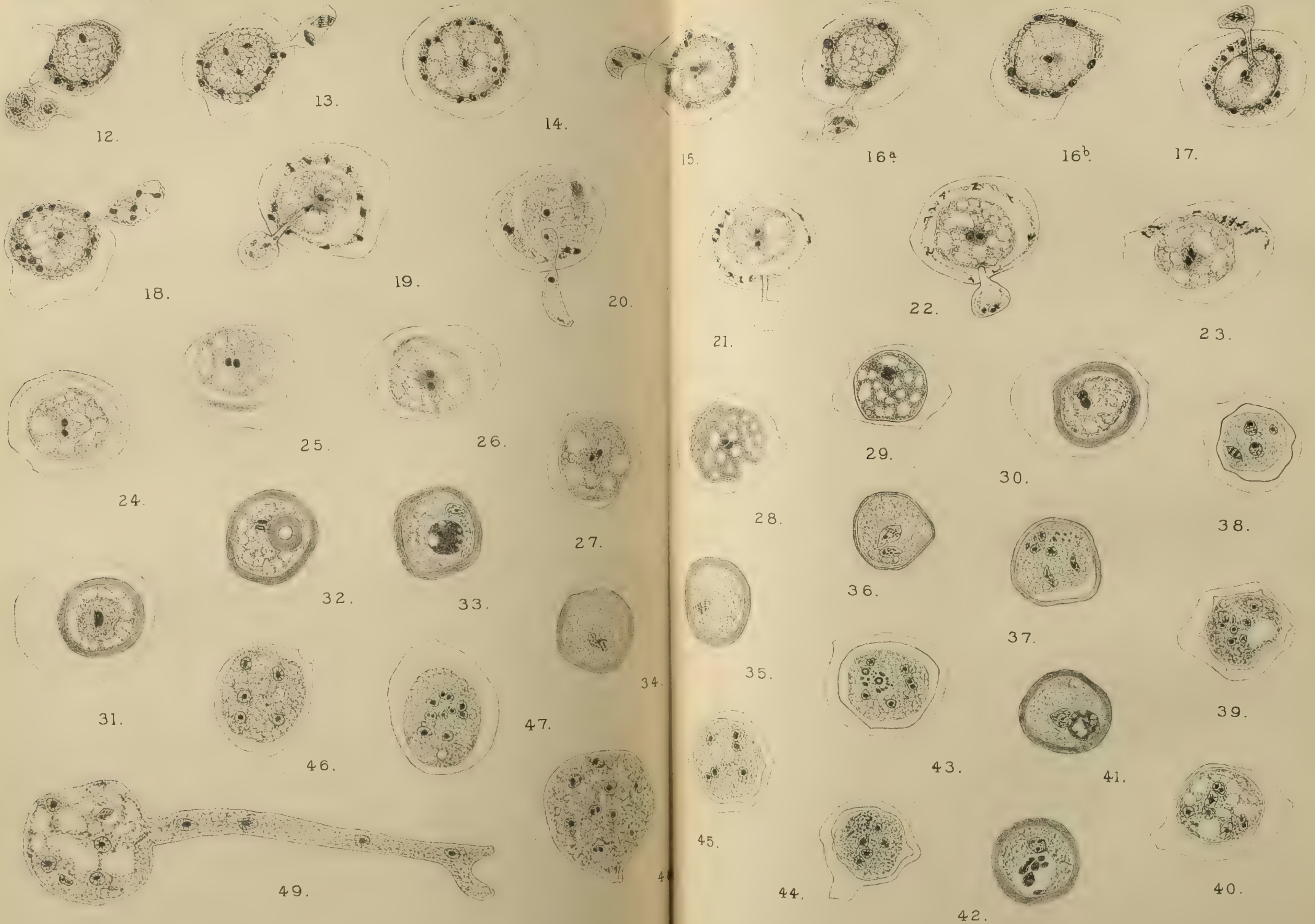
TROW.—PYTHIUM.

University Press Oxford.



A.H.Trow, del.





A.H. Trow, del.

University Press, Oxford.

Researches on Coprophilous Fungi.

BY

GEORGE MASSEE, F.L.S.,

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AND

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—♦—

With Plates XVII and XVIII.

—♦—

UNTIL recently the systematic side of Mycology has received most attention in Britain, a fact which probably accounts for the absence of any specific work on Coprophilous or dung-borne Fungi, many of which, as the species of *Gymnoascus*, *Thelebolus*, *Microascus*, &c., on account of being the most primitive representatives of their respective groups, are perhaps more interesting from a morphological than a purely systematic standpoint. A second reason may lie in the fact that many of the species are so very minute that their presence cannot be detected in the field; in fact it is only after considerable experience that they can be seen under the most favourable conditions of illumination, and as their duration is in many instances ephemeral the only chance of success is by keeping the material upon which they grow in the laboratory, where a daily examination can be made. Even from a systematic standpoint our investigations have shown that a careful study of the Fungi growing on the dung

[Annals of Botany, Vol. XV. No. LVIII. June, 1901.]

of various animals will in all probability add many interesting species to our Mycologic Flora.

In many continental countries this special branch of Mycology has not been neglected, excellent work having been done by Hansen (15) in Denmark; Boudier (4) and Crouan (11, 12) in France; Coemans (8, 9) and Marchal (20) in Belgium; Spegazzini (37) in Italy; Heimerl (17) and Zukal (50-54) in Austria; Winter (41-45) and Zopf (47-49) in Germany; Karsten (19) in Finland, and Chelchowski (7) in Poland.

MORPHOLOGY, SPORE-GERMINATION, ETC.

As coprophilous Fungi do not form a concrete group they cannot be treated from a comparative point of view, nevertheless certain structural features claim attention. The most typical of ascigerous coprophilous Fungi are those included in the *Sordarieae* and the *Ascoboleae*, and as already noted of dung-borne Fungi, when treating of the genus *Coprinus* in this Journal (22), the spores in both these groups are as a rule comparatively large and deeply coloured—characteristics of a primitive type; in the genera *Ascobolus* and *Saccobolus* the spores—as in every Fungus—are hyaline when young, subsequently the epispore passes from pale lilac through bright violet to deep brown, the final colouration in many instances becoming so intense as to render the spore opaque when viewed by transmitted light. In the *Sordarieae* the spores are always dark brown and often quite opaque—hence appearing black at maturity.

In some species of *Ascobolus* each spore is surrounded by a hyaline mucilaginous layer, whereas in *Saccobolus* the four or eight spores contained in an ascus are agglutinated together at maturity, in a definite manner for each species, by a mass of mucilage (Figs. 50, 51). This massing together of the spores in the ascus is the one feature that distinguishes the genus *Saccobolus* from *Ascobolus*. It is, however, in species of the *Sordarieae* that the presence of hyaline mucilage on the spores reaches its fullest development. In many species the

mucilage forms a uniform sheath enclosing the entire spore (Figs. 9, 10), appearing under the microscope as a hyaline refractive peripheral belt or zone, varying in width in different species. In other species of *Sordaria* the spores first appear as eight hyaline vermiform bodies each nearly as long as the ascus (Fig. 8). At a later stage a swelling appears near the apex of each spore (Fig. 8); this swelling continues to increase in size and absorbs the protoplasm from the slender portion below, which is eventually cut off by a septum from the swollen portion or spore proper. Eventually the spore becomes coloured, while the basal portion remains colourless and forms an appendage. Appendages formed in this manner have a cellulose wall, and in rare instances the septum cutting off the cavity of the spore from that of the appendage is not developed, when the wall of the latter becomes coloured like that of the spore, thus proving that the colouration of the epispore depends on the presence of living protoplasm in the cell. We have observed this phenomenon repeatedly in *Sordaria globosa*, and it has also been noted by Hansen (15) in an undetermined species of *Sordaria*.

In addition to the appendage described above, a second kind consisting of mucilage is also often present. Such an appendage may be attached to the apex or base, or to both ends of the spore (Fig. 15), or even to the end of the primary appendage which is cut off from the young spore as described above (Fig. 16). These mucilaginous appendages usually disappear when the spore reaches maturity; they become much swollen and eventually deliquesce in contact with water, and stain readily with methylene blue. In the two species of *Xylaria* growing on dung the spores are of very large size and surrounded by a broad zone of mucilage, whereas in the numerous other species of *Xylaria* growing on wood, &c., the spores are usually minute and always destitute of a mucilaginous external layer.

Zopf (48) considers that the mucilaginous appendages of the spores are formed from the protoplasm in the ascus not used up in the formation of the spores, and also suggests that

the appendages serve to keep the spores in their relative positions in the ascus, and states that in some *Sordarieae* the uppermost spore is attached to the apex of the ascus.

In species of *Ascobolus*, *Ryparobius*, *Saccobolus*, *Sordaria*, and *Thelebolus* we have observed that at maturity the spores are ejected in an agglutinated mass, often to a considerable distance. This ejection occurs equally in bright sunshine or in darkness. The sudden shooting out of the spores from the ascus appears to depend on the absorption of moisture by the mucus and consequent increase of volume. When a section of an ascophore containing mature spores is examined in the dry state no change occurs, but when water is applied the mucus in the asci is seen to swell and the spores are suddenly shot forth. If alcohol is used instead of water the spores do not move. In the species of *Chaetomium* and in *Spumatoria* the asci deliquesce and the mucus thus formed swells by the absorption of water and escapes, carrying the spores along with it, through the mouth of the perithecium, where it forms a ball which persists for some time until dissolved by rain or dew.

Attempts on the part of De Bary (13), Brefeld (6), and Janczewski (18) to germinate *Ascobolus* spores resulted in failure, and the opinion is generally entertained that a passage through the intestinal canal of an animal is necessary to induce germination. The last-named author fed rabbits with bread containing *Ascobolus* spores, and observed that germination had commenced when the dung was deposited. Our experiments have proved that *Ascobolus* spores will germinate without passing through the intestinal canal, nevertheless we believe that the majority of the spores of species of *Ascobolus* that eventually produce fruit on dung have been swallowed along with food by herbivorous animals.

When the spores of species of *Ascobolus* are ejected, the mucus that holds them in a group sets firmly immediately on exposure to the air into a mass almost insoluble in water. For the purpose of obtaining pure sowings of spores, dung, bearing Fungi of the species required, was enclosed in a Petri

dish. At the expiration of two or three hours, numerous groups, each containing eight spores, were deposited on the lid of the dish, and so firmly cemented that they could not be removed with a needle without breaking the mass to powder. When such pure deposits of spores are secured it is only necessary to add a nutritive medium for the purpose of studying germination. When spores so deposited have been immersed for a week in liquid they still remain in groups firmly cemented to the glass. The spore-masses ejected by *Thelebolus stercoreus* behave in a similar manner.

The above experiments prove that the wholesale diffusion of *Ascobolus* spores by wind is out of the question, and the explanation of the presence of species of *Ascobolus* on practically every portion of dung deposited seems to be that the spores from the Fungi on a given piece of dung are ejected and alight on the surrounding grass. Such spore-laden grass is eaten by some herbivorous animal, its dung in turn produces more Fungi, which in due course diffuse their spores, and thus the continued production of the Fungus is secured.

The following indirect evidence of the method of spore diffusion by herbivorous animals supports the previous statement. An analysis of the Fungi occurring on dung as given by Saccardo (34) gives the following figures:—On dung of herbivora, 708 species; on carnivora, 45 species; on reptilia, 4 species. Moreover, when the dung of a herbivorous animal is cut into pieces it is found that the cut surfaces yield a crop of Fungi quite equal in number to those produced on the original external surface. The only means of accounting for the growth of Fungi in the position thus described is on the supposition that the spores were swallowed along with the food.

We succeeded in germinating the spores of *Ascobolus perplexans* and a form of *A. glaber* with dull white apothecia (*A. albidus*, Crouan) under the following conditions. One sowing was made in a hanging-drop of tap-water, another in a drop of decoction of dung, average temperature 80° F. After twenty hours nearly all the spores showed vigorous germ-tubes, frequently proceeding from both ends of the

spore. These germ-tubes at the end of two days had grown to strong branched long hyphae (Fig. 56). The exospore showed a tendency to break up in strips, as is figured by Janczewski (18) for the germinating spore of *Ascob. furfuraceus*. In a control experiment with spores of the same form of *A. glaber* at a temperature of about 60° F. not a single spore had germinated at the end of twenty hours, although subsequently slight germination occurred in many of the spores. As illustrative of the readiness with which the spores of this white form of *A. glaber* germinate, the following fact may be mentioned. Some rabbit-dung, bearing a rich crop of the above *Ascobolus*, was kept in a tin box. As the asci became ripe the spores were thrown on to the side of the box, where they germinated, and formed a mycelium which eventually produced apothecia. In a second set of experiments, with spores of *A. perplexans* in a hanging-drop of dung decoction at 80° F., germination was vigorous; but under the same conditions as above, except at 60° F., no trace of germination occurred. In a hanging-drop of dung decoction with addition of one per cent. of pepsin, temperature 85° F., germination was very vigorous. In a second culture similar to the last in constituents, but at 65° F., there was a mere trace of germination. As a set-off against the above our failures to induce germination of the spores of other species of *Ascobolus* and those of other dung Fungi were numerous, and the only point that appeared in the series of experiments was that the higher the temperature the greater the success in securing germination. This is what would be expected if it is an advantage that spores should pass through the alimentary canal of an animal, although we are inclined for the following reasons to look upon this factor as acquired.

The spores of certain species of *Ascobolus* that do not grow on dung, but on wood, decaying vegetable matter, or on the ground, germinate readily under artificial conditions, as shown by Boudier (4), whereas, as already stated, the spores of dung-borne species as a rule do not germinate at all under similar conditions; nevertheless these highly specialized dung species

sometimes revert more or less to the habits of the more primitive species yet growing on the ground, thus betraying their origin.

A quantity of dung bearing a rich growth of *Ascobolus glaber* was kept shut up in a tin box. The mature spores of the Fungus had been ejected and adhered to the lid and sides of the box, where they germinated and produced in due course a plentiful crop of ascophores, which were somewhat paler in colour than the typical form from which they originated: furthermore, the mucilage, which is abundant and of a pale brown colour in the typical form, was very scanty and colourless. Spores obtained from the typical Fungus growing on dung could not be induced to germinate, whereas the spores of the retrogressive form grown on the lid of the box germinated in a hanging-drop of dung decoction at 80° F., but would not germinate at a lower temperature. Spores of the Fungus growing on the box lid were received on the lid of a Petri dish and in turn produced ascophores, growing in a decoction of plum-jam in gelatine. The spores of these Fungi, representing the second generation removed from the typical form, germinated readily in water at a temperature of 60° F. The spores of this last generation were passed through the intestinal canal of a guinea-pig, and it was found that no germination had taken place; the spores also refused to germinate afterwards in a decoction of dung at a high temperature.

Eurotium insigne, originally discovered on dung by Winter in Germany, has been met with in great abundance on kangaroo-, horse- and fowl-dung at Kew. Its very large, globose, spinulose spores germinated vigorously in a decoction of dung, soon forming a webbed mycelium which on the fifth day produced the conidial condition of the Fungus—a member of the form-genus *Gliocladium*—and on the eleventh day the young ascigerous condition appeared, reaching maturity in five more days.

Thelebolus stercoreus is of special interest on account of the important position assigned to it by Brefeld in his classification of Fungi. In Brefeld's scheme *Thelebolus* is placed in the

class *Hemiasci*, of which it alone constitutes the division *Carpohemiasci*, and from it Brefeld considers that the whole of the *Carpoasci*—*Discomycetes* and *Pyrenomycetes*—are derived. His principal reasons for so doing are based on the variability shown in the size of the organ containing the spores—called an ‘ascus-like sporangium’ by Brefeld—as also in the number of the contained spores. These characters, however, are not more pronounced in *Thelebolus* than in *Ryparobius*, which Brefeld admits as a true *Discomycete*; in fact, in many genera having polysporous asci both in the *Discomycetes* and *Pyrenomycetes* the variability in the size of the ascus and in the number of the contained spores is as great as in *Thelebolus*: *Ryparobius*, *Comesia*, *Tromera*, *Fracchiæa*, and *Coronophora* are examples of such genera (cf. Figs. 46, 47).

A distinction of primary importance overlooked by Brefeld is found in the fact that a sporangium is multinucleate from the first, whereas an ascus is at first uninucleate. The spore-containing organ in *Thelebolus* is at first uninucleate. Further, at maturity the spores are ejected in a mass through a definite opening at the apex, exactly as is the case with the asci of some species of *Ryparobius*; whereas in the *Zygomycetes* the spores escape through the irregularly ruptured wall of the sporangium, as in *Mucor*, or the sporangium is shot off with its contained spores, as in *Pilobolus*. For the reasons given above we consider the spore-containing organ in *Thelebolus* to be a typical ascus, and shall speak of it under that name in future.

Brefeld considers that the parenchymatous wall completely surrounding the ascus in *Thelebolus* is homologous with the web of vegetative hyphae at the base of the sporangiophore in *Mortierella Rostafinskii*, and that the long stem-like sporangiophore in the last-named species is represented in a much reduced condition as a single large cell at the base of the ascus in *Thelebolus*. Respecting the covering of the ascus in *Thelebolus*, it may be said that morphologically it agrees in every detail with the protective portion known as the ascophore in *Ryparobius*; it also agrees with the undifferen-

tiated hyphae present in *Mortierella Rostafinskii* in originating at a point situated below the specialized reproductive portion of the Fungus—the point of origin common to protective structures in all Fungi. As to the homology of the single large cell, described and figured by Brefeld (5), at the base of the ascus in *Thelebolus* with the sporangiophore in *Mortierella*, we may state that an examination of serial microtome sections of *Thelebolus* does not support this view. The sections show that there exists, not a single cell as stated by Brefeld, but a group or row of large cells representing the ascogonium of the *Pezizeae*. Owing to the exceedingly minute size of the young ascophore, orientation for the purpose of sectioning is practically impossible. Our sections, although not enabling us to figure the organ in question in detail, prove the existence of some multicellular structure resembling an ascogonium.

Brefeld states that only one ascus is present in an ascophore, and that when two or more asci appear to be present this is due to the growing together of contiguous originally distinct ascophores. In many ascophores of *Thelebolus* only one ascus is present; serial microtome sections, further, show that contiguous ascophores may adhere to each other; other sections also show that from one to four asci may be present in the same ascophore. In some species of *Ryparobius* the asci are constantly very few in number, and the structure of the ascophore in such cases is identical with the ascophores of *Thelebolus* containing several asci. It may be pointed out, also, that in this matter of the variability of the number of asci *Thelebolus* resembles the genus *Sphaerotheca* (*Erysiphaceae*), where as a rule only a single ascus is found in each perithecium, but where in some instances two or three asci occur. In such cases in both *Thelebolus* and *Sphaerotheca* the asci are much smaller than the normal solitary ascus.

In conclusion, we fail to detect any evidence of affinity with the *Zygomycetes*, but on the other hand consider *Thelebolus* as very closely allied, if even distinct as a genus, from *Ryparobius*. In this view we are supported by Heimerl (17), Schroeter (35), and Rehm (31).

DISTRIBUTION.

Coprophilous Fungi are somewhat numerous; Saccardo (34) enumerates 757 species included in 187 genera. Of these, many, so far as is known, occur only on dung; others again, especially those belonging to the *Hyphomycetes*, are not so strictly confined to dung but may also occur on decaying vegetable matter. Too little is known as yet of the dung-flora of many countries to admit of any comparison being made as to relative numbers in different regions or as to the range of individual species, nevertheless records from such localities as Algeria, S. & E. Africa, Martinique, Ceylon, Borneo, Australia, Tasmania, Tonkin, Malacca, Spitzbergen, United States, Canada, Cuba, Cayenne, Argentina, and Patagonia, show that coprophilous Fungi are widely distributed. Rostrup (33) records no less than seven species of *Sporormia*, besides species of *Saccobolus*, *Ascophanus*, and *Raparobius* from Greenland. As already stated, the great majority of coprophilous Fungi occur on the dung of herbivorous animals, hence their general distribution will be influenced by the relative number of such animals in a given region.

In addition to the examination of a large quantity of dung of various native and domestic animals from different localities in Britain, we have been enabled, through the kindness of Mr. C. Bartlett, Superintendent of the Zoological Gardens, Regent's Park, to examine the dung of a number of exotic animals, and we find that as a rule the various species of Fungi are not confined to one specific habitat, e.g., certain species, as *Gymnoascus Reessii*, *Eurotium insigne*, *E. microsporum*, *Sporormia longipes*, and others grow indiscriminately on the dung of any herbivorous animal, and often appear as a successive wave on isolated portions of different kinds of dung. Many different Fungi not unfrequently flourish on the same substratum; seventy-two species have been recorded as growing on rabbit-dung.

When quite fresh dung is placed under a bell-jar the

sequence of development is as follows: first, *Phycomycetes*, usually heralded in by *Pilaira* and *Pilobolus*, followed by species of *Mucor*, accompanied by their parasites *Chaetocladium*, *Thamnidium*, *Piptocephalis*, *Syncephalis*, &c.; next appear various members of the *Hyphomycetes*, probably in many instances representing the conidial condition of the ascigerous Fungi which are usually the last in the sequence to appear.

We have found that dung can be kept under observation for several months if placed in a shallow vessel on a layer of blotting-paper kept constantly moist, and covered with a bell-jar; a small quantity of naphthaline serves to check the ravages of various mites, eelworms, &c.

SYSTEMATIC.

We give below an account of the species which appeared on dung under observation during the six months Oct.–March. Only those Fungi belonging to the *Ascomycetes* are enumerated here, the *Phycomycetes* and *Hyphomycetes* being reserved for a future paper. Summarizing the results from a systematic standpoint, we may note the occurrence of two new genera, *Pleuroascus* (*P. Nicholsoni*), belonging to the *Perisporiaceae*, and *Spumatoria* (*S. longicollis*) to the *Sphaeriaceae*. New species of the following genera have appeared: *Endomyces coprophilus*, *Eurotium microsporum*, *Magnusia Bartlettii*, *Sordaria globosa*, *Sporormia longipes*, *Microascus variabilis*, *M. nidicola*, and *Melanospora discospora*. The following species are new to the British Flora: *Myxotrichum uncinatum*, *Thelebolus stercoreus*, *Ryparobius ascophanoides*, *Eurotium insigne*, *Sordaria anserina*, *S. minima*, *S. hirta*, *S. setosa*, *S. curvicolla*, *S. pleiospora*, *S. macrospora*, *S. neglecta*, *S. Winteri*, *S. fimiseda* var. *appendiculata*, *Delitschia moravica*, *D. insignis*, *Sporormia ovina*, *S. pulchella*, *S. fime-taria*, *Sphaeroderma fimbriatum* and *S. Hulseboschii*. The genus *Bovilla* Sacc., founded on the single (British) species *B. Caproni*, proves to be nothing more than the immature condition of *Sordaria coprophila*.

A fact worthy of note is the occurrence on the dung of exotic animals from the Zoological Gardens, London, of many British species common on the dung of our native and domestic animals. As instances of this may be mentioned the occurrence in abundance of *Ascophanus equinus* on the dung of Elephant and Mexican Deer; *Ascobolus immersus* on the dung of Grys-bok, Sinaitic Ibex, &c.; *Sordaria fimicola* on the dung of Elephant and Kangaroo; *S. curvula* on the dung of Elephant, Giraffe, and Mexican Deer; *Sporormia minima* on the dung of Giraffe and Dorcas Goat.

It is obvious therefore that, in many cases at least, no character of specific value can be attached to the occurrence of a species on the dung of any particular animal. It may be remarked, also, that in the *Sordarieae* the superficial or immersed position of the perithecium is to a large extent determined by the hard or soft texture of the dung on which it is growing.

ASCOMYCETES.

Gymnoascaceae. *Endomyces coprophilus*, sp. nov. (Fig. 35).

Hyphis arachnoideis irregulariter ramosis crebro septatis albis 4–6 μ crassis, ascis lateralibus brevissime stipitatis piriformibus vel globulosis 4–8-sporis 20–30 \times 18–25 μ , sporis ellipticis hyalinis 5–6 \times 3–3.5 μ .

Hab.—In fimo equino, Kew, 1894 and Oct. 1900.

On account of the scattered asci the present Fungus is for the time being placed in the genus *Endomyces*, although it shows little relationship with any of the described species of this genus. In the present plant the creeping mycelium is very delicate and slender, and never becomes concentrated to form a patch visible even under a strong lens,—hence the species is met with only by chance, and intermixed with other Fungi. The Fig. 45, p. 12, in Mass. Brit. Fung. Fl., by mistake referred to *Gymnoascus Reessii*, represents the present species.

Arachniotus ruber (van Tiegh.), Schroet. in Cohn's Krypt.-Fl. Schles., Bd. iii, Hälfte 2, 211 (1893).

Gymnoascus ruber, van Tiegh. in Bull. Soc. Bot. France, xxiv, 159 (1877); Sacc. Syll. Fung. viii, 823 (1889); Mass. Brit. Fung. Fl. iv, 19 (1895).

Hab.—On the dung of Burrhel Wild Sheep (*Ovis burrhel*), and not uncommon on dog's dung, Kew, Feb.–Mar. 1891.

Gymnoascus Reessii, Baran. in Bot. Zeit., xxx, 158, Taf. III, A (1872); Sacc. Syll. Fung. viii, 823 (1889); Mass. Brit. Fung. Fl. iv, 19 (excluding Fig. 45, p. 12) (1895).

Hab.—On the dung of Red Deer (*Cervus elaphus*), Sinaitic Ibex (*Capra sinaitica*), Roe (*Capreolus caprea*), Giraffe (*Camelopardalis giraffa*), Kangaroo (*Macropus giganteus*) and Rabbit, Kew, Nov.–Mar. 1901.

Myxotrichum uncinatum (Eidam), Schroet. (Figs. 30–32).

Gymnoascus uncinatus, Eidam, in Cohn's Beitr., Bd. iii, 293 (1880); Wint. in Rabenh. Krypt.-Fl. Deutschl., Bd. i, Abth. 2, 16 (1887); Sacc. Syll. Fung. viii, 824 (1889).

Myxotrichum uncinatum (Eidam), Schroet. in Cohn's Krypt.-Fl. Schles., Bd. iii, Hälfte 2, 212 (1893).

Tufts rounded, gregarious, about $\frac{3}{4}$ mm. across, at first sulphur-yellow, then becoming dark yellow, and finally reddish, hyphae interwoven, much branched, usually at right angles, septate, towards the interior colourless and ascigerous, at the periphery anastomosing, reddish brown, with numerous long ($120-200 \times 5-6 \mu$) orange or reddish-orange 1-septate or aseptate branches, which at maturity are uncinuate at the tip; asci very numerous, subglobose, $8-9 \mu$ in diameter, borne laterally in dense clusters on colourless hyphae towards the centre of the tuft, wall very evanescent, 8-spored; spores minute laterally compressed, in front view subglobose, $2.5-4 \mu$ in diam., in side view ellipsoidal, yellowish, minutely asperous.

Hab.—On Rabbit-dung, Kew, Dec. 1900; on dung of Patagonian Cavy (*Dolichotis patachonica*), Kew, Feb. 1901.

(Distrib.—Germany, on dung of Mouse, Sparrow, and 'carnivorous animals.')

Each tuft is more or less globular in shape, and is composed of a mass of interwoven hyphae. These hyphae are coloured at the periphery, and frequently anastomose, forming a network-like structure, which bears numerous long uncinuate branches or appendages; towards the interior of the ball-like mass the hyphae are colourless and much more delicate, and give origin to an immense number of minute asci. The wall of the ascus is more or less mucilaginous and very evanescent, so that the free spores are found in a dense mass towards the interior of each tuft. Frequently however the spores, after the deliquescence of the ascus-membrane, are held together by mucilage for some time in groups of eight. The spores, under a magnification of

670 diam., are seen to be distinctly rough with minute scattered points.

The two examples recorded above differ slightly from one another. In the specimens on Rabbit-dung the appendages are slightly narrower, and the spores smaller, measuring (seen from the front) $2.5-3\mu$ in diam.; in the specimens on the dung of Patagonian Cavy the appendages are a little stouter, and the spores larger, although somewhat variable in size, measuring $3-4\mu$. In both examples the spores are minutely asperous, and the plants indistinguishable in general habit.

Ascobolaceae. *Thelebolus stercoreus*, Zukal (Figs. 41-44, 66).

T. stercoreus, Zukal, in Denkschr. d. Math.-Nat. Klasse, Kais. Acad. d. Wissensch. Wien, li, Abth. 2, 21, Taf. I (1886); Heimerl, in 15. Jahresber. d. k. k. Ober-Realsch., Bezirk Sechsh., Wien, 28 (1889); Brefeld, Unters. a. d. Gesamtgeb. d. Myk., ix, 113, Taf. III, A (1891); Sacc. Syll. Fung. x, 34 (1892); Schroet. in Cohn's Krypt.-Fl. Schles., iii, pt. 1, 51 (1893); Rehm in Rabenh. Krypt.-Fl. Deutschl., Bd. 1, Abth. 3, 1106 (1896).

Ryparobius monoascus, Mouton, in Bull. Soc. Roy. Bot. Belg., xxv, pt. 1, 141 (1886); Sacc. Syll. Fung. viii, 545 (1889).

Thelebolus nanus, Heimerl, l. c., 30, Taf. I, f. 2 (1889); Sacc. Syll. Fung. x, 34 (1892); Rehm, l. c., 1107 (1896).

Apothecia scattered or subgregarious, very variable in size, $135-240\mu$ in diam., at first closed, globose to ovoid, pallid, becoming dull brownish, glabrous, semi-immersed, wall parenchymatous, very delicate and thin, composed of irregularly shaped polygonal cells $6-14\mu$ wide, apothecia at length ruptured at the apex by the protruding ascus; ascus solitary, or 2-3, rarely as many as 5, ovate-oblong to broadly ovate, variable in size, $120-230 \times 80-170\mu$, polysporous; spores $5-7 \times 3-3.5$, oblong, rounded at each end, hyaline, smooth, ejected in a mucilaginous mass to some distance, paraphyses very delicate, hyaline, filiform, sometimes branched, closely surrounding the ascus, soon disappearing.

Hab.—Abundant on Rabbit-dung, Kew and Reigate, England, Nov. 1900 and Mar. 1901; on Horse-dung, Kew, Nov. 1900.

(Distrib.)—Austria and Belgium; on dung of Hare, Rabbit, Goat, Roe- and Red-Deer.)

The question of the affinity of this interesting Fungus has already

been discussed at pp. 319-321, and it may simply be remarked here that structurally it is to be considered as a *Ryparobius* containing as a rule only a single very large ascus, but sometimes varying with 2-3, or even 5 asci. Zukal (l. c.) identified the present species as the *Thelebolus stercoreus* of Tode (Fungi Mecklenb. select., fasc. 1, 41 (1790)), and this determination has been followed by other authors. A comparison, however, of the characters given by Tode (l. c.) with those of the present plant quite precludes, in our opinion, the supposition that the two plants are identical; e.g. Tode specially characterizes his species as being of a bright yellow colour, the words 'croceus' and 'aureus' being used. Saccardo (Syll. Fung. x, 34), adopting the name *Thelebolus stercoreus*, Tode, for Zukal's plant, places the genus among the *Ascomycetes*; it is to be noted, however, that *Thelebolus terrestris*, Alb. and Schw., is a true *Gasteromycete*.

T. nanus was separated from *T. stercoreus* by Heimerl on account of its smaller size, but in examining a large series of growing *T. stercoreus* it becomes clearly evident that *T. nanus* is nothing more than a small form, as a complete series of intermediates can be found growing together. *T. nanus* has already been recorded as British by Smith (36).

Associated with normal specimens of *T. stercoreus* (on rabbit-dung, Kew) we met with a Fungus quite similar to that described as *Ascozonus oligoascus* by Heimerl (17), except that in our plant we were not able to observe the 'elongate-clavate' yellow hairs which, according to Heimerl, crown the young apothecia. Our Fungus, which is represented at Fig. 45 (cf. Heimerl, l. c., Fig. 1, a), appeared to be nothing more than the form of *T. stercoreus* containing 2-3 asci.

***Ryparobius ascophanoides*, Sacc. (Figs. 33-34).**

Ascophanus ryparobioides, Heimerl, Oesterr. Ascob., 22, f. xi (1889).

Ryparobius ascophanoides, Sacc. Syll. Fung. x, 33 (1892); Rehm, in Rabenh. Krypt.-Fl. Deutschl., Bd. i, Abth. 3, 1101 (1896).

Scattered, very minute, $\frac{1}{4}$ - $\frac{1}{8}$ mm., white, almost hyaline when moist, sessile on a broad base, disk slightly concave, glabrous; asci few, 6-8, broadly clavate, $160 \times 60 \mu$, 32-spored; spores elliptic, ends obtuse, smooth, $15-16 \times 8-9 \mu$; paraphyses filiform, apex not thickened, septate, about 2μ thick.

Hab.—On rabbit-dung, Kew. (Distrib.—Austria, on Deer's dung.)

Allied to *R. sexdecimsporus* (Cr.), Boud., differing in the broadly clavate asci, containing thirty-two spores. The above measurements

of asci and spores are taken from the British specimens. Heimerl (l. c.) gives the size of asci and spores as follows: asci, $120-174 \times 50-73 \mu$; spores, $14.5-17.5 \times 7-8.5 \mu$. In the British specimens each ascus contained thirty-two spores; Heimerl states that this is the usual number for the present species, although he has observed cases of the occurrence of twenty-nine and about forty-five spores in an ascus. In *A. sexdecimsporus* the normal number of spores in each ascus is sixteen; it may be worth recording here, however, that in one instance we observed eighteen fully-formed spores to be present.

Ascophanus microsporus (Berk. and Br.), Phill.; Mass. Brit. Fung. Fl. iv, 173 (1895).

Hab.—On the dung of Argali Sheep (*Ovis ammon*), Kew, Nov. 1900.

A. equinus, Mass. Brit. Fung. Fl. iv, 179 (1895); Boud., Mém. Ascob. 254, Pl. 12, xlii-xliv (1869).

Hab.—On the dung of Mexican Deer (*Cariacus mexicanus*) and Elephant (*Elephas africanus*), Kew, Dec. 1900.

A. ochraceus (Cr.), Boud., Mém. Ascob. 247, Pl. II, xxxiv (1869); Mass. Brit. Fung. Fl. iv, 176 (1895).

Hab.—On the dung of Burrhel Wild Sheep (*Ovis burrhel*), Mar. 1901.

Ascobolus perplexans, sp. nov. (Figs. 52-55).

Apotheciis sparsis luteo-virentibus extus minutissime furfuraceis mox glabris piriformibus, disco initio concavo demum plano vel subconvexo, acute marginatis $0.5-1$ mm. latis; ascis cylindræis basi attenuatis octosporis $190-210 \times 16-20 \mu$; sporis oblongo-ovatis monostichis $18-19 \times 9-10 \mu$, episporio longitudinaliter rimuloso et parce reticulato demum verruculoso violaceo; paraphysibus filiformibus, interdum ramosis, sursum incrassatis septatis sæpe nodulosis muco flavovirente obvolutis.

Hab.—In fimo *Raphiceri melanotidis* (Grys-bok), *Elephantis africani* (Elephant), *Macropodis gigantei* (Kangaroo), *Cervi elaphi* (Red Deer), *Ovis vignei* (Ural Wild Sheep), Kew, Nov.-Feb. 1901; in fimo equino, S. Kensington, Mar. 1901.

A very beautiful species, perhaps most nearly allied to *A. glaber*, Pers. The episporium is at first simply longitudinally cracked, but at a later stage it becomes broken up in an areolate manner, the small portions of episporium resembling minute warts under a magnification of 400 diam.

A. vinosus, Berk., Boud., Mém. Ascob. 221, Pl. 6, xi. (1869), Mass. Brit. Fung. Fl. iv, 159 (1895).

Hab.—On Goose-dung, Kew, Dec. 1900.

A. immersus, Pers.; Boud., Mém. Ascob. 226, Pl. 8, xvii (1869), Mass. Brit. Fung. Fl. iv, 168 (1895).

Hab.—On dung of Grys-bok (*Raphicerus melanotis*), Sinaitic Ibex (*Capra sinaitica*), Red Deer (*Cervus elaphus*), and Ural Wild Sheep (*Ovis vignei*), Kew, Nov.–Dec. 1900.

Saccobolus quadrisporus, sp. nov. (Figs. 48–51).

Apotheciis $\frac{1}{2}$ – $\frac{2}{3}$ mm. diam., primo plus minus convexulis diaphanis aquoso-violaceis demum planis opacis atro-violaceis vel nigrescentibus, maturitate disco ob ascos exsiliantes brunneo-punctato, ascis subcylindraceis sursum truncatis, deorsum breviter stipitatis saepe curvatis $95\text{--}110 \times 18\text{--}20 \mu$, jodo caerulescentibus quadrisporis; paraphysibus simplicibus vel furcatis filiformibus apice clavulatis septatis; glomerulis sporidiorum ellipticis $40\text{--}45 \times 15\text{--}16 \mu$, sacculo communi hyalino inclusis, sporidiis in una vel quibus seriebus ordinatis atro-violaceis, $20 \times 8\text{--}10 \mu$, praesertim ad margines verrucis minutis praeditis.

Hab.—In fimo anserino, Royal Gardens, Kew, Nov. 1900.

A congeneribus ascis quadrisporis distinguenda.

The apothecia of the present species are at first of a clear watery violet colour, but gradually become with age less translucent and darker in colour. At maturity the disk is studded with the apices of the protruding asci, and at this stage, if the ascophore is placed in a drop of water, the ripe asci eject their spores, still surrounded by their 'sac,' into the water. The outline of this 'sac,' or mucilaginous covering, round the ejected spores, is almost invisible without staining, but on using methylene blue the mucus becomes clearly visible. A considerable number of ascophores were examined, and in every case the asci were found to be tetrasporous. The spores when separated appear somewhat trigonous in shape; when seen in the clustered position (Fig. 51) each spore shows a pale line running across it, which is apparently due, not to a crack in the epispore, but to a greater translucency in this part, caused by the shape of the spore. The epispore is broken up into the wart-like bodies only on the free side of each spore. The ascospores are usually arranged in two parallel rows as shown at Fig. 51; not unfrequently, however, the four spores are found arranged side by side in one row.

S. Kerverni (Cr.), Boud., Mém. Ascob. 229, Pl. 8, xviii (1869); Mass. Brit. Fung. Fl. iv, 171 (1895).

Hab.—On Horse-dung, Reigate, Surrey, Dec. 1900; on dung of Mexican Deer (*Cariacus mexicanus*) and Dorcas Goat (*Capra dorcas*), Kew, Dec. 1900.

S. depauperatus (Berk. and Br.), Rehm; Mass. Brit. Fung. Fl. iv, 170 (1895).

Hab.—On dung of Mexican Deer (*Cariacus mexicanus*), Kew, Feb. 1901.

S. neglectus, Boud., Mém. Ascob. 231, Pl. 9, xx (1869); Mass. Brit. Fung. Fl. iv, 171 (1895).

Hab.—On dung of Ural Wild Sheep (*Ovis vignei*), Kew, Feb. 1901.

Pezizaceae. Helotium lacteum (Cke and Phill.), Mass. Brit. Fung. Fl. iv, 269 (1895).

Hab.—On Rabbit-dung, Kew, Dec. 1900.

Perisporiaceae. Pleuroascus, gen. nov.

Perithecia subiculo intertexto pannoso distincto vel confluyente insidentia, atra, astoma, membranaceo-carbonacea, fragilia, contextu parenchymatico, appendicibus pluribus hyalinis arcte spiraliter convolutis instructa; ascis globosis minutis numerosissimis mox diffluentibus in hyphis ramosis pleurogenis; sporis minutis fuligineis globosis.

The present genus appears to be distinct among the Phaeosporae of the Perisporiaceae in the dense pannose subiculum and the minute globose laterally-borne asci. The asci are produced in great numbers, and arise in a glomerulate manner on the delicate branched hyphae which fill the perithecium. This latter character suggests affinity with *Cephalotheca*.

P. Nicholsoni, sp. nov. (Fig. 25).

Peritheciis in subiculo pannoso distincto vel confluyente primum albo deinde rufescente e hyphis delicatulis ramosis intertextis composito plus minus immersis, dense gregariis, parvis, 130–200 μ diam., atris, fragilibus, contextu parenchymatico e cellulis distinctis 6–10 μ latis composito, appendicibus pluribus e parte peritheci inferioris orientibus hyalinis arcte spiraliter convolutis compressis septatis; ascis numerosissimis minutis globosis 8 μ diam., mox diffluentibus, in hyphis ramosis dense glomerulatis; sporis octono-conglobatis, globosis, minutis, circ. 3 μ diam., fuligineis.

Hab.—In fimo *Caviae cobayae* (Guinea Pig), Kew, England, Jan. 1901 (coll. Geo. Nicholson).

The dense pannose subiculum, in which the perithecia are at first

more or less completely immersed, is when young of a pure white colour, but finally becomes at the time of the maturity of the perithecia of a dull rusty colour. It may in advanced age disappear altogether, leaving the perithecia exposed, but held together in masses by the interwoven elastic spiral appendages. The appendages remain permanently hyaline, and closely spirally wound. They are compressed and ribbon-like in structure and arise from the equatorial and basal cells of the external wall of the perithecium. The appendages when drawn out are seen to be very long, many times exceeding the diameter of the perithecium. The spores remain for some time hyaline, but become finally distinctly fuliginous.

Eurotium insigne, Wint. (Figs. 29, 39-40).

E. insigne, Wint., in Rabenh. Fung. eur., nr. 1732 (1874); Wint. in Rabenh. Krypt.-Fl. Deutschl., Bd. i, Abth. 2, 61 (1887).

Penicillium insigne (Wint.), Schroet., in Cohn's Krypt.-Fl. Schles., Bd. iii, 220 (1893).

The conidial form of this species is *Gliocladium penicilloides*, Corda, Icon. Fung. iv. 31, Pl. VII, f. 92 (1840); Grove, in Journ. of Bot. xxiii, 165, tab. 256, f. 9 (1885); Sacc. Syll. Fung. iv, 85 (1886); Massee, Brit. Fung. Fl. iii, 293 (1893); Matruch., in Rev. génér. d. Bot. vii, 322, Pl. XVI, ff. 1-10 (1895).

G. macropodium, March., in Bull. Soc. Roy. Bot. Belg. xxxiv, pt. 1, 135, Pl. I, f. 6, a, b (1895); Sacc. Syll. Fung. xiv, 1048 (1900).

Mycelium inconspicuous, perithecia large, $\frac{1}{2}$ -1 mm. in diam., superficial, globose, glabrous, when young clear white, then pallid, becoming yellowish, and finally rusty-brown, thin-walled, membranaceous, wall composed of large polygonal or irregularly-shaped cells 15-20 μ wide; asci numerous, subglobose or globose-pyriform, with a short stalk, 45-65 \times 38-45 μ , very evanescent, 8-spored; spores large, globose, 17-20 μ diam., covered all over with short acute spines, at first colourless, becoming distinctly grey.

Hab.—On dung of Kangaroo (*Macropus giganteus*), Kew, Jan. 1901; on dung of Burrhel Wild Sheep (*Ovis burrhel*), Mär. 1901; on dung of Fowls, Kew, Jan. 1901; on Horse-dung (originally from Epping Forest), Kew, Feb. 1901. (Distrib.—(of ascigerous form), Germany and France; on dung of Goose and Dog.)

A remarkable and very distinct species in its large size and strongly spinous spores, which finally become distinctly grey in colour. Winter, in first describing the *Eurotium* in Rab. Fung. Eur., remarked that

Gliocladium penicilloides, Corda, which was observed by him closely associated with it, probably represented the conidial stage. In 1895, Matruchot (l.c.) in cultivating *Gliocladium penicilloides* obtained ascigerous perithecia, which, from the description given, were clearly those of *Eurotium insigne*, Wint. Matruchot, sowing the ascospores obtained in this way, found that they produced a mycelium which gave rise to the conidiophores of the *Gliocladium*. In our examples, recorded above, on the dung of Fowls, Kangaroo, and Horse, the *Eurotium* was in each case preceded by the *Gliocladium*, and we were able to observe clearly the organic continuity of the two forms, the same mycelium which produced conidiophores bearing the young perithecia. Culture experiments were also made, with the result of confirming fully Matruchot's observations. Ascospores were sown on sterilized films of cork floating on a decoction of dung. These ascospores germinated readily, producing a mycelium which at the end of five days gave rise to typical conidiophores of *Gliocladium penicilloides*. After eleven days the same mycelium produced perithecia (containing young asci) of *Eurotium insigne*.

The *Gliocladium*, recorded as a new species under the name of *G. macropodium*, by Marchal, on the dung of Kangaroo, is evidently not distinct from *G. penicilloides*, the distinguishing character relied upon, viz. the greater length of the conidia—'9-11 μ long' instead of '6 μ long,' being quite insufficient. In our specimens, on the dung of Kangaroo, &c., the conidia varied from 7-12 μ in length; Matruchot, also, expressly states that the size of the conidia is extremely variable in the species of *Gliocladium*, and gives 5-10 μ as the length of the conidia in *G. penicilloides*.

Grove (l.c.) remarks that *Gliocladium* differs from *Penicillium* in the spores being produced singly, not in chains; this statement, however, is erroneous, as in both genera the spores are originally produced in chains, but in *Gliocladium*, as Matruchot points out, the spores soon lose this arrangement, and become irregularly massed in the mucilaginous head. Grove, also, referred doubtfully the *Penicillium*-like fungus represented by Cooke in Plowright's Monograph of the British *Hypomyces* (Grevill. xi, 49, Pl. 156) as occurring associated with *Hypomyces aureo-nitens*, Tul., to *Gliocladium penicilloides*, and for this reason the statement occurs in Mass. Brit. Fung. Fl. iii, 294, that *G. penicilloides* 'is considered to be the conidial stage of *Hypomyces aureo-nitens*.' It is quite clear, however, that the Fungus

occurring with the *Hypomyces* was not *Gliocladium*, as the characteristic mucilaginous covering of the heads was absent.

On account of the characters shown in its conidial stage, and on account of the dark-coloured ascospores, it seems doubtful if the present Fungus should remain in the genus *Eurotium*; it should perhaps, as Winter and Matruchot think, be made the type of a new genus.

E. microsporum, sp. nov. (Fig. 28).

Peritheciis parvulis sparsis circ. 130μ diam. globosis primo flavo-virentibus dein citrinis superficialibus contextu parenchymatico e cellulis tenuibus $5-8\mu$ latis composito; ascis sphaeroideis vel subsphaeroideis $7-8\mu$ diam., citissime diffluentibus, octosporis; sporis minutissimis sphaeroideo-biconvexis 2μ diam. laevibus chlorinis.

Hab.—In fimo *Capreoli capraeae* (Roe), *Haplocerotis montani* (Rocky Mountain Goat), *Caprae dorcadis* (Dorcas Goat), *Caprae sinaiticae* (Sinaitic Ibex), *Ovis vignei* (Ural Wild Sheep), *Caviae cobayae* (Guinea Pig), Kew, Jan.—Feb. 1901.

The present species is remarkable for the small size of the ascospores, and also for the manner in which they become exposed at maturity. The wall of the perithecium, which is at first sage-green in colour and then yellowish, becomes dry and very brittle when the perithecia are quite ripe, and completely falls away, exposing the chrome-yellow mass of spores. The perithecia occur singly, dotted at intervals on the dung, and when crushed under the microscope are seen to be filled with innumerable minute free ascospores. Except in the early stages of the development of the perithecium it is difficult to find any trace of asci, as the walls of the latter deliquesce almost immediately. The spores viewed from the front are roundish in outline; from the side, biconvex.

Magnusia Bartlettii, sp. nov. (Fig. 26).

Peritheciis sparsis superficialibus globosis nigris $\frac{1}{4}-\frac{1}{2}$ mm. diam., contextu parenchymatico e cellulis minutis polygonis distinctis circ. 4μ latis composito, apice coma pilorum ornatis, pilis $8-12$ rigidis simplicibus plus minus divergentibus atro-fuscis laevibus sparse septatis apice interdum flexuosis basin versus circ. 6μ latis perithecii diametro duplo vel triplo longioribus, caeterum glabris; ascis numerosis globosis ($18-20\mu$ diam.) vel oblongo-piriformibus ($20-25 \times 14-17\mu$) $6-8$ -sporis, cito diffluentibus; sporis ellipticis utrinque acutis $8-10 \times 5\mu$, primum hyalinis demum distincte dilute fuliginis.

Hab.—In fimo *Capreoli caprae* (Roe), *Caviae cobayae* (Guinea Pig), *Ovis burrhel* (Burrhel Wild Sheep), *Ovis vignei* (Ural Wild Sheep), Kew, Jan.—Feb. 1901.

The above species appears to be distinct from *M. nitida*, Sacc.—the only other species in the genus—in the simple, not uncinatè, appendages, which are narrower and more flexuose. It appeared first on the dung of an albino Roe, sent from the Zoological Gardens by Mr. Bartlett.

Sphaeriaceae. *Chaetomium elatum*, Kze; Cooke, Handb. Brit. Fung. 652 (1871); Zopf, Entw. Ascomycet. (*Chaetomium*), 83 (1881). *C. comatum* (Tode), Fr., Sacc. Syll. Fung. i, 221 (1882).

Hab.—On dung of Red Deer (*Cervus elaphus*), Kew, Dec. 1900.

C. murorum, Corda; Cooke, Handb. Brit. Fung. 653 (1871); Zopf, Entw. Ascomycet. (*Chaetomium*), 80, Taf. VI, ff. 13–20 (1881); Sacc. Syll. Fung. i, 223 (1882).

Hab.—On dung of Red Deer (*Cervus elaphus*), Roe (*Capreolus caprae*), Dorcas Goat (*Capra dorcas*), Sinaitic Ibex (*C. sinaitica*), Burrhel Wild Sheep (*Ovis burrhel*), and Ural Wild Sheep (*O. vignei*), Kew, Jan.—Mar. 1901.

Sordaria globosa, sp. nov. (Fig. 21).

Peritheciis subgregariis primo subimmersis dein plus minus superficialibus globosis circ. $\frac{3}{4}$ mm. diam. glabris olivaceis, ostiolo papilliformi atro subcarbonaceo, contextu parenchymatico e cellulis tenuibus composito; ascis numerosis cylindraceutis deorsum in stipitem longum attenuatis octosporis $300-350 \times 35-50 \mu$; sporis ellipticis $35-40 \times 20-22 \mu$, basi tantum appendiculo hyalino cylindraceuto saepe attenuato persistente recto vel curvulo $25-30 \mu$ longo auctis.

Hab.—In fimo *Macropodis gigantei* (Kangaroo) et *Cervi elaphi* (Red Deer), Kew, Feb. 1901.

Peritheciis globosis glabris et sporis majusculis basi tantum caudatis mox dignoscenda species.

The Fungus described above is well marked in the globose perithecia, which are of a rather pale olivaceous colour, with a very dark brown or black papilliform ostiolum, and the large spores, each with a single basal appendage. The latter is a true cell, with a distinct cell-wall.

S. anserina (Rabenh.), Wint. (Fig. 6).

Malinvernia anserina, Rabenh. in Herb. Myc., nr. 526.

Sordaria anserina, Wint., Deutsch. Sordar., 99, Taf. XI, Fig. 20 (1873); Sacc. Syll. Fung. i, 238 (1882); Wint. in Rab. Krypt.-Fl. Deutschl., Bd. i, Abth. 2, 173 (1887).

Perithecia gregarious, up to $\frac{1}{3}$ mm. high, semi-immersed, blackish-brown, rugulose, basal part subglobose narrowed upwards into a long or short curved rather thick bluntly conical neck, which bears a tuft of hairs below the apex on one side, hairs bristle-like long rigid dark brown, each usually composed of a fascicle of slender hyphae, 100–250 (rarely 350) μ long, 3.5 μ wide; asci cylindrical, attenuated below into a rather long pedicel, about $280 \times 25\text{--}30 \mu$, 4-spored; spores uniseriate, ovate to broadly ovate, $36\text{--}44 \times 18\text{--}22 \mu$, provided at the more or less truncate basal end with a definite hyaline appendage about equalling the spore in length.

Hab.—On Goose-dung, Royal Gardens, Kew, England, Nov. 1900; on dung of Giraffe (*Camelopardalis giraffa*) and Kangaroo (*Macropus giganteus*), Kew, Dec. 1900.

(Distrib.)—Germany and N. Italy; on dung of Goose, Cow, Sheep, Horse, and Rabbit.)

S. anserina is easily distinguished among the *Sordariae* by its perithecia possessing towards the base of the curved beak, on one side only (the convex side), a conspicuous tuft of bristle-like hairs, and by its 4-spored asci. Winter records cases where only two spores occurred in the ascus; in such cases the ascus is much shorter than the normal tetrasporous one, while each of the two spores is twice the usual size. The external perithecial wall of *S. anserina* appears frequently to have a rather curious structure, being irregularly marked with peculiar dark-coloured thickened portions on a pale membrane, and so is not truly cellular. The same structure is often found in *S. setosa*, Wint. The spores when young have appendages at both ends, but at maturity the basal end only, which is more or less truncate, possesses a rather thick appendage about equalling the length of the spore.

S. minima, Sacc. and Speg. (Fig. 11).

S. minima, Sacc. and Speg., in Mich. i, 373 (1878); Fung. Ital. t. 617 (1879).

Hypocopra minima, Sacc. Syll. Fung. i, 244 (1882).

Perithecia more or less superficial, very small, about $100 \times 80 \mu$, broadly ovate, glabrous, perithecial wall membranous, thin, very pale brown or almost colourless, translucent, cells about 5 μ wide; asc few,

cylindrical, shortly stalked, $45-60 \times 8-10 \mu$, 8-spored: spores uniseriate, ovoid, $4.5-8 \times 3-4 \mu$.

Hab.—On Rabbit-dung, Royal Gardens, Kew, England, Oct. 1900; on Hare-dung, Kew, Mar. 1901. (Distrib.—N. Italy; on Cow-dung.)

S. minima has hitherto been known from only a single locality in Italy. The species is at once recognized by the small size of all its parts, and being almost invisible under a simple lens, is probably often passed over. Saccardo and Spegazzini give the size of the spores as $8 \times 4 \mu$, but in our specimens one perithecium contained asci with spores measuring only $4.5 \times 3 \mu$; in other instances, however, the spores measured $6-7 \times 3.5-4 \mu$. The wall of the perithecium is so translucent that the asci and coloured spores can be seen within the closed perithecium.

S. hirta, Hans. (Fig. 7).

S. hirta, Hans., in Vidensk. Meddel., 1876, p. 336, Pl. VII, ff. 17-24 (1876-77); Sacc. Syll. Fung. i, 232 (1882).

Perithecia rather large, $1-1\frac{1}{2}$ mm. high, basal part subglobose, olivaceous, narrowed upwards into a rather long, blackish, conical neck, which is more or less covered with dark brown or nearly black septate rigid simple hairs; asci long-stalked, clavate-fusoid; spores 8 (rarely 4), ovoid, $40-46 \times 20-22 \mu$, furnished at both ends with a hyaline appendage about equalling the spore in length.

Hab.—On Cow-dung, Kew, England, Nov. 1900. (Distrib.—Denmark, on Cow-dung.)

Hansen (l.c.) remarks that the size of the spores is extremely variable in the present species, and records cases of spores varying between the wide limits of $24-58 \times 13.5-25 \mu$.

S. setosa, Wint. (Fig. 13).

S. setosa, Wint., Deutsch. Sordar. 97, Taf. X, f. 18 (1873); Schroet. in Cohn's Krypt.-Fl. Schles., Bd. iii, Hälfte 2, 288 (1894).

Philocopra setosa, Sacc. Syll. Fung. i, 249 (1882).

Podospira setosa, Wint., in Rabenh. Krypt.-Fl. Deutschl., Bd. i, Abth. 2, 176 (1887).

Perithecia scattered or subgregarious, $\frac{1}{3}-\frac{1}{2}$ mm. high, greenish black, often surrounded at the base with spreading pallid hyphae, subimmersed or wholly superficial, subglobose with a short or rather long bluntly conical blackish neck, which is sometimes slightly curved, neck and sometimes the upper part of perithecium more or less covered with long stiff greyish acute mostly aseptate bristles, usually

about 150μ long, but varying in length from $50-300\mu$, bristles sometimes arranged in tufts; asci about 15, $250-300 \times 50-60\mu$, broadly cylindrical or ventricose, narrowed towards the apex, shortly stalked, wall very thin, soon deliquescent, 128-spored; paraphyses multiseptate, filiform, about equalling the asci; spores greenish-black, ellipsoid, $18-20 \times 10-11\mu$ (collected into a mass measuring about $250 \times 55\mu$), at first with appendages at both ends, but eventually with a single straight hyaline appendage at the basal end only, usually slightly shorter than the spore.

Hab.—On Rabbit-dung, Kew, England, Jan. 1901; on dung of Giraffe (*Camelopardalis giraffa*), Red Deer (*Cervus elaphus*), Mexican Deer (*Cariacus mexicanus*), Sinaitic Ibex (*Capra sinaitica*), Dorcas Goat (*C. dorcas*), Ural Wild Sheep (*Ovis vignei*), Kew, Nov. 1900–Feb. 1901. (Distrib.—Germany; on dung of Goose and Sheep, also cultivated on Horse- and Cow-dung, and on paper saturated with dung.)

The present species is easily distinguished from most of the *Sordariae* by its many-spored asci; from *S. pleiospora*, Wint., described below, it differs in its more hairy perithecium, and the more numerous much smaller spores; from *S. curvicolla*, Wint., it is distinguished by its slightly larger spores and by the ascus being narrowed towards the apex (Fig. 13). The spores at a certain stage in their development possess thread-like appendages, often apparently of indefinite length, at each end, but when the spores are mature, or nearly so, only a single appendage, about 3μ wide and slightly shorter than the spore, is found at the basal end. Winter (Deutsch. Sordar. (l.c.)) figures and fully describes the development of the spores. Zukal (53) states that the stiff bristle-like hairs surrounding the ostium first appear towards the end of the period of the ejaculation of the spores.

S. curvicolla, Wint. (Fig. 12).

S. curvicolla, Wint., in Hedwigia, x, 161 (1871); Wint., Deutsch. Sordar. 98, Taf. X, f. 19 (1877); Speg. in Michel. i, 228 (1878).

Philocopra curvicolla (Wint.), Sacc. Syll. Fung. i, 250 (1882).

Podospora curvicolla, Wint., in Rabenh. Krypt.-Fl. Deutschl., Bd. i, Abth. 2, 176 (1887).

Perithecia scattered, immersed to the base of the neck, basal part ovate-globose narrowed upwards into a rather long thick obtuse neck, wall parenchymatous, upper part of perithecium, including the neck,

more or less covered with short hairs; asci ventricose or ovate-saccate, shortly pedicellate, not narrowed upwards but broadly rounded at the apex, about 128-spored, $270-350\ \mu$ long, about $120\ \mu$ broad; spores ellipsoid, $15-16 \times 9-10\ \mu$, with a hyaline cylindrical basal appendage, slightly shorter than the spore.

Hab.—On Rabbit's dung, Kew, England, Feb. 1901. (Distrib.—Germany and N. Italy; on dung of Mouse, Rabbit, and Hare.)

S. curvicolla is distinguished from *S. setosa*, Wint.,—to which it is closely allied,—by its smaller spores, and especially by the shape of its ascus, which is broadly rounded at the apex, not narrowed upwards.

A form of the present species, with 150 spores in the ascus, is recorded by Griffiths (Bull. Torr. Bot. Club, xxvi, 437, Pl. 365, ff. 13-15 (1899)) from the United States, growing with *Sordaria curvula* on *Salsola kali*, var. *tragus*.

S. pleiospora, Wint. (Fig. 14).

S. pleiospora, Wint., in Hedwigia, x, 161 (1871); Wint., Deutsch. Sordar., 93, Taf. X, f. 17 (1873); Schroet. in Cohn's Krypt.-Fl. Schles., Bd. iii, Hälfte 2, 288 (1894).

Philocopra pleiospora, Sacc. Syll. Fung. i, 249 (1882).

Podospora pleiospora, Wint., in Rabenh. Krypt.-Fl. Deutschl., Bd. i, Abth. 2, 175 (1887).

Perithecia dark brown or blackish, up to 1 mm. high, scattered, at first subimmersed, but becoming finally wholly superficial, basal part subglobose narrowed upwards into a thick bluntly conical neck, which is nearly glabrous, lower part of perithecium provided with rather long scattered soft flexuous greyish-brown septate hairs, cells of perithecial wall small, $6-7\ \mu$ wide; asci broadly cylindrical or ventricose, narrowed towards the apex, about $300 \times 60-80\ \mu$, 16-64-spored; spores ovoid, somewhat truncate at one or both ends, $25-30 \times 15-18\ \mu$, at first with an appendage at each end, but at maturity losing both appendages.

Hab.—On dung of Giraffe (*Camelopardalis giraffa*), Kew, Jan. 1901. (Distrib.—Germany, N. Italy, Poland; on dung of Horse, Cow, and Hare.)

Easily distinguished from *S. setosa* and *S. curvicolla* by the fewer and larger spores. These three species—*S. pleiospora*, *S. setosa*, and *S. curvicolla*—are by some authors placed in a separate genus, *Philocopra*, founded solely on the polysporous asci. This one character,

however, cannot be considered sufficient to separate the species from *Sordaria*, and it seems better, with Schroeter (l. c.), to treat the distinction as having a sectional value only within the genus.

Griffiths (Bull. Torr. Bot. Club, xxvi, 438 (1899)) records a *Sordaria* under the name of *S. pleiospora* from the United States, but the characters given differ so much in important points from those of the present species that it seems very doubtful if *S. pleiospora* was really found.

The spores are recorded by most authors as varying from 16-64, and Winter states that the latter is the highest number reached; in our examples, however, asci containing 76 spores were observed.

S. macrospora, Auersw. (Fig. 9).

S. macrospora, Auersw., in Rab. Fung. Eur., nr. 954; Niessl. Beitr., p. 30, Tab. VI, f. 43 (1872); Wint., Deutsch. Sordar., 79, Taf. VII, f. 4 (1873); Wint. in Rabenh. Krypt.-Fl. Deutschl., Bd. i, Abth. 2, 165 (1887); Schroet. in Cohn's Krypt.-Fl. Schles., Bd. iii, Hälfte 2, 286 (1894).

Hypocopa stercoris, Fckl., Symb. Myc. 241 (1869).

H. macrospora, Sacc. Syll. Fung. i, 241 (1882).

Perithecia black, gregarious or crowded, glabrous, semi-immersed or entirely superficial, $\frac{1}{3}$ - $\frac{1}{2}$ mm. in height, basal part subglobose narrowed upwards into a short bluntly conical neck, perithecial wall parenchymatous, cells 10-20 μ wide; asci elongate-cylindrical, 210-300 \times 18-30 μ , tapering below into an evident stalk, apex rounded, thickened, 8-spored; spores uniseriate, broadly obovate or oblong, rounded at the apical end, and acutely and minutely pointed at the basal end, 24-31 \times 15-18 μ , surrounded by a narrow layer of mucilage.

Hab.—On dung of Hare (*Lepus europaeus*) and Kangaroo (*Macropus giganteus*). Kew, Dec. 1900. (Distrib.—Finland, Germany, Poland, N. Italy, on dung of Hare, Sheep, Mouse, Rabbit, Horse, and Cow.)

S. macrospora is easily recognized by the peculiarly incrassate apex of the ascus and the broad spores minutely pointed at the basal end. The latter character, which has not apparently hitherto been noted, is found also in the spores of the closely related *S. fimicola* (Rob.), Ces. and De Not., which differs in the smaller, narrower spores, surrounded by a wider layer of mucilage.

On Hare-dung the perithecia are more or less immersed, while on Kangaroo-dung they are entirely superficial.

S. neglecta, Hans. (Fig. 15).

S. neglecta, Hans., in Vidensk. Meddel., 1876, p. 335, Tab. IX, ff. 12-18 (1876-77); Sacc. Syll. Fung. i, 232 (1882).

Perithecia rather large, about 1 mm. high, semi-immersed, basal part subglobose, narrowed upwards into a short blackish bluntly conical or papilliform neck; asci longly cylindrical or somewhat fusiform, narrowed into a long stalk, rounded at apex, $400-500 \times 35 \mu$, 8-spored; spores uniseriate, broadly ovoid, $40-50 \times 25-29 \mu$, provided at each end with a permanent hyaline usually curved appendage, about equalling the spore in length.

Hab.—On Cow-dung, Reigate, England, Nov. 1900. (Distrib.—Denmark, on Cow- and Horse-dung.)

A fine and apparently uncommon species, distinguished by its large spores with two permanent appendages.

S. Winteri, Karst. (Fig. 5).

S. Winteri, Karst., Myc. Fenn. ii, 251 (1873); Sacc. Syll. Fung. i, 234 (1882).

S. breviseta, Karst. (nec Fckl.), Myc. Fenn. ii, 52 (1873).

Perithecia scattered or gregarious, at first immersed, becoming free, $\frac{1}{3}$ mm. high, basal part subglobose, olivaceous, narrowed upwards into a short conical blackish neck, which is more or less covered with short, scattered, rigid, acute, septate dark brown hairs; asci cylindrical-clavate, pedicellate, sporiferous part about $150 \times 30-40 \mu$, 8-spored; spores ellipsoid, $23-25 \times 15-16 \mu$, provided at the basal end with a hyaline appendage slightly shorter than the spore.

Hab.—On Horse-dung, Kew Green, England, Dec. 1900. (Distrib.—Finland, on dung of Horse, Cow, and Hare.)

S. fimiseda, Ces. et De Not., var. **appendiculata** (Auersw.) (Fig. 16).

S. appendiculata, Auersw., Niessl. Beitr., p. 38, Tab. V, f. 40 (1872); Sacc. Syll. Fung. i, 234 (1882).

S. fimiseda, Ces. et De Not., Wint. in Hedwigia, xiii, 55, f. v (partim) (1874); Wint., Deutsch. Sordar. 89, Taf. IX, f. 13 (partim) (1877).

Podospora fimiseda, var. *appendiculata* (Auersw.), Wint. in Rabenh. Krypt.-Fl. Deutschl., Bd. i, Abth. 2, 170 (1887).

Perithecia scattered or gregarious in small clusters, superficial, ovate-conical in general outline, basal part ovate-globose narrowed upwards into a short thick usually curved neck, blackish-brown, basal part of perithecium covered with long soft flexuose spreading hairs, which are

fuliginous, septate, and about $2-3\ \mu$ wide, neck, especially at apex, densely covered with similar but shorter and erect hairs, wall of perithecium composed of small cells about $5\ \mu$ wide; asci cylindrical, about $220 \times 30-35\ \mu$, gradually attenuated into a long stalk, 8-spored; spores broadly elliptical, $28-30 \times 19-20\ \mu$, appendiculate at each end, basal appendage persistent, rather stout, shorter than the spore ($15-18\ \mu$), bearing at the free end a secondary flexuose narrower appendage, $50-60\ \mu$ long, which soon disappears, apical appendage longer than the spore, evanescent.

Hab.—On dung of Mexican Deer (*Cariacus mexicanus*), Kew, Jan. 1901. (Distrib.—Germany, on dung of Hare.)

As Winter points out, the present Fungus differs from *S. fimiseda*, Ces. and De Not., only in the smaller spores, which are about half the size of those of that species. If this difference were constant, it might be considered to afford a specific character, but Winter expressly states that he has found spores intermediate in size. It seems best, therefore, to place the present plant as a variety under *S. fimiseda*—the nature and development of the appendages of the spore being quite similar in both cases. As Winter (Deutsch. Sordar. p. 90) has remarked, the basal appendage, which appears first, represents a true cell, and is quite different in origin from the two secondary appendages: ‘Dass diese Anhängsel auch morphologisch verschieden sind, wurde schon gesagt; das an der Basis der Spore direct ansitzende ist eine specifische Zelle, die beiden anderen offenbar nur gallertartig quellende Verdickungen der Membran der Spore und des Hauptanhängsels.’

The example here recorded on the dung of Mexican Deer differs apparently from the usual form, which is described as ‘covered all over with short septate hairs,’ in the longer denser hairs of the perithecium, and the compact apical tuft.

Sordaria sp. (Fig. 17).

A remarkable form, or species, of *Sordaria* occurred on Kangaroo-dung exposed for some months to the open air. The perithecia were black, rather large ($\frac{3}{4}$ –1 mm. high, $\frac{1}{2}$ mm. wide), densely crowded, and held together at the base by a bysoid stroma composed of brown interwoven hyphae. The most remarkable feature, however, was the wall of the perithecium, the external cells of which were nearly all transformed into broad-based spine- or prickle-like structures, each composed of a single cell. Each prickle-like cell (Fig. 17) measured

from $30-60\mu$ across the base. Only a few spores were seen, measuring $20-22 \times 10-12\mu$, and provided at one end with a rather long hyaline appendage. The asci seen were few, and ill-developed, and it seems therefore possible that the present form is to be regarded as a monstrous development of some species of *Sordaria*, induced by unfavourable (abnormal) conditions. It may be mentioned that the Kangaroo-dung was fully exposed to the effects of the weather, and for a few days was buried under a heavy fall of snow.

S. fimicola (Rob.), Ces. and De Not.; Wint., Deutsch. Sordar. 81, Taf. VII, f. 6 (1873); Sacc. Syll. Fung. i, 240 (1882), sub *Hypocopra*.

Hab.—On the dung of Elephant (*Elephas africanus*), Hare (*Lepus europaeus*), and Kangaroo (*Macropus giganteus*), Kew, Dec. 1900.

On the Elephant's dung the perithecia are quite superficial, and only very loosely attached to fragments of straw, &c. The layer of mucilage surrounding the spores (which are minutely pointed at one end just as in *S. macrospora*, Auersw.) is usually rather wide, measuring $4-5\mu$ across at each side of the spore.

S. platyspora, Plowr., in Grevill. vi, 28, Tab. XCIV, f. 2 (1877); Sacc. Syll. Fung. i, 241 (1882), sub *Hypocopra*.

Hab.—On Horse-dung, Kew Green, and Hastings, England, Dec. 1900.

The Kew specimens are remarkable for the irregularity in the shape of some of the ascospores, which instead of showing the normal almost circular form (when seen from the front), have a nearly square or irregularly waved outline.

S. curvula, De Bary; Wint., Deutsch. Sordar. 101, Taf. XI, f. 22 (1873); Sacc. Syll. Fung. i, 233 (1882).

Hab.—On the dung of Mexican Deer (*Cariacus mexicanus*), Giraffe (*Camelopardalis giraffa*), and Elephant (*Elephas africanus*), Kew, Oct. 1900.

On the dung of the first-named animal the perithecia were quite superficial, and attached by a dense web of colourless hyphae.

S. minuta, Fckl.; Wint., Deutsch. Sordar. 100, Taf. XI, f. 21 (1873); Sacc. Syll. Fung. i, 231 (1882).

Hab.—On Rabbit-dung, Kew, Dec. 1900, with tetrasporous asci; on the dung of Mexican Deer (*Cariacus mexicanus*) and Sinaitic Ibex (*Capra sinaitica*), Kew, Jan. 1901, with octosporous asci; on the dung of Kangaroo (*Macropus giganteus*), Kew, Feb. 1901,—a form with eight somewhat lemon-shaped, inaequilateral spores.

S. decipiens, Wint., Deutsch. Sordar. 92, Taf. IX, f. 16 (1873); Sacc. Syll. Fung. i, 235 (1882).

Hab.—On Rabbit-dung, Kew, Dec. 1900; on the dung of Giraffe (*Camelopardalis giraffa*), Mexican Deer (*Cariacus mexicanus*), and Sinaitic Ibex (*Capra sinaitica*), Kew, Dec. 1900.

The Kew examples are very interesting on account of the variation shown in the number of spores in the ascus. *S. decipiens* has been described by all authors as having 8-spored asci; in some of the Kew examples, however, perithecia occurred in which all the asci contained sixteen spores. It was quite clear, moreover, that the same species, viz. *S. decipiens*, was under observation, as in one perithecium, from another gathering on Rabbit-dung, two asci each containing sixteen spores were present among the normal octosporous ones. In Saccardo's 'Sylloge' only the species of *Sordaria* with four and eight spores in the ascus are included under that genus, the species with 'polysporous' asci (i.e. asci containing sixteen or more spores) being removed to another genus, *Philocopra*. Such specific variation as that recorded above shows the artificiality of genera founded merely on the number of spores in the ascus.

S. stercoraria (Sowerb.).

Sphaeria stercoraria, Sowerb., Brit. Fung., t. 357 (1803).

Hypocopra stercoraria, Sacc. Syll. Fung. i, 244 (1882).

An examination of the type specimen at Kew shows that in this species the spores, which are elliptical and laterally compressed, and surrounded by a layer of mucilage, measure $45-60 \times 22-38 \mu$. The measurement—'30 μ long'—given by Saccardo, therefore, requires correction.

S. coprophila (Fr.), Ces. and De Not.

Sphaeria bovilla, Cooke, Handb. Brit. Fung., ii, 874, Fig. 394 (1871).

Bovilla Capronii, Sacc. Syll. Fung. ii, 360 (1883).

The Fungus called by Cooke *Sphaeria bovilla* has been made by Saccardo the type of a new genus *Bovilla*. Examination of Cooke's material (now in the Kew Herbarium) shows, however, that the Fungus is merely the immature stage of *Sordaria coprophila*. The genus *Bovilla*, being founded on this single specimen, must therefore be abolished.

Delitschia moravica, Niessl. (Fig. 18).

D. moravica, Niessl., Notiz. Pyren. 47, Taf. IV, f. 22 (1876); Sacc. Syll. Fung. i, 733 (1882); Wint. in Rabenh. Krypt.-Fl. Deutschl., Bd. i,

Abth. 2, 179 (1887); Schroet. in Cohn's Krypt.-Fl. Schles., Bd. iii, Hälfte 2, 290 (1894).

Perithecia minute, about $\frac{1}{4}$ mm. high, at first subimmersed, but becoming almost free, subgregarious, subglobose, with a short thick blunt blackish neck, which is covered with short scattered rigid dark setae; asci narrowly cylindrical, $170-200 \times 12-18 \mu$; spores obliquely 1-seriate or sometimes irregularly biseriate, oblong or ellipsoid, straight, constricted at the septum, provided at each end with a minute subhyaline wart-like apiculus, $20-22 \times 8-9 \mu$, surrounded by a narrow layer of mucilage.

Hab.—On Rabbit-dung, Reigate, England, Nov. 1900. (Distrib.—Austria-Hungary, on dung of Hare.)

The bristly perithecia and the wart-like apiculus of the spores distinguish the present species from the commoner *D. minuta*, Fekl.

D. insignis, Mout. (Fig. 20).

D. insignis, Mout., in Bull. Soc. Roy. Bot. Belg. xxxvi, part 2, 13, t. A, ff. 7, 8 (1897); Sacc. Syll. Fung. (Supp.) xiv, 558 (1900).

Perithecia about 1 mm. high, more or less scattered, immersed, glabrous, basal part globose-ovate, olivaceous, narrowed upwards into a rather long, cylindrical, stout, blackish-brown neck; asci elongate-cylindrical, $300-320 \times 25-30 \mu$, rounded at the apex, gradually narrowed below into the stalk, 8-spored; paraphyses septate, filiform, often branched, longer than the asci; spores biseriate, oblong, somewhat rounded at the ends, deeply constricted and eventually separating at the septum, $44-67 \times 10-16$ (rarely 20) μ , provided at each end with a hyaline straight or flexuous appendage about equalling the spore in length.

Hab.—On Horse-dung, Epping Forest, Essex, Oct. 1900, and Kew, Nov. 1900. (Distrib.—Belgium, on Cow-dung.)

We are indebted to Prof. Saccardo for the identification of this species. *D. insignis* is a fine and well-marked species, and it is somewhat remarkable that it has for so long escaped notice. The plant was originally discovered by Mouton (l.c.) in 1897, on Cow-dung, in Belgium, and this remained the only locality known until in November last we met with the species on Horse-dung in Epping Forest. It is possible that *D. insignis* may occur not uncommonly in this country, as a search on Horse-dung at Kew at once resulted in finding the species. *D. insignis*, together with another species—*D. sordarioides*, Speg., from S. America—are peculiar in the genus in possessing caudate appendages in the

place of the layer of mucilage which surrounds the spores of most species of *Delitschia*. On account of this difference the question has been raised as to whether these two species can be placed in the genus *Delitschia*. There seems, however, every reason for doing so when we remember that we find in the related genus *Sordaria* certain species (*S. macrospora*, *S. fimicola*, &c.) with spores surrounded by a layer of mucilage, while other species have one or two caudate appendages to their spores. Mouton (l.c.) observes of the spores of *D. insignis*, after describing them as 'medio septata,'—'nonnunquam ad quartam partem inferiorem tenuiter (spurie?) septata.' In our specimens, however, the only septum observed was at the middle, where the spore is much constricted. At this place the spore, at maturity, falls readily into two halves, each of which may for a little time retain its tail, but the latter finally becomes completely absorbed, and each half of the spore appears then as a more or less elliptical cell.

D. Winteri, Plowr. (Fig. 22).

D. Winteri, Plowr., in Grevill. ii, 188, Tab. XXV, f. 1 (1874); Wint. in Hedwigia, xiii, 52, f. 3 (1874); Sacc. Syll. Fung. i, 734 (1882).

Hab.—On Rabbit-dung, Reigate, England, Nov. 1900. (Distrib.—England, N. Italy, Belgium, on dung of Rabbit, Hare, Cow, and Sheep.)

This species does not seem to have been hitherto met with in England, since it was originally discovered by Plowright at King's Lynn, Norfolk, in 1873. The following diagnosis is drawn up from our specimens: Perithecia scattered, almost completely immersed, up to 1 mm. high, $\frac{1}{2}$ – $\frac{1}{3}$ mm. broad, glabrous, basal part ovate-globose, olivaceous, narrowed upwards into a thick bluntly conical blackish brown neck, cells of perithecial wall 10–15 μ wide; asci large, elongate-cylindrical, about 500 μ long and 40–45 μ wide, narrowed below into a short stalk, 8-spored; spores obliquely uniseriate, broadly oblong, obtuse at both ends, slightly constricted at the septum, opaque, dark-fuscous, 50–60 \times 25–28 μ , surrounded by a layer of mucilage. *D. Winteri* agrees so closely in nearly all its characters with *D. Auerswaldii*, Fckl., that it is possible that the two are not specifically distinct. In both we find the large spores (variable in length), slightly constricted at the septum, and bluntish at the ends, the only difference being that in *D. Winteri* the spores are from 25–28 μ wide, while in *D. Auerswaldii*, judging from the examples in Fckl., Fung. Rhen. 2034, they appear to average about 20 μ . It must be mentioned,

however, that many authors describe the spores of *D. Auerswaldii* as narrower than this, Winter giving the measurement 16μ , Saccardo 18μ , and Schroeter $17-21\mu$.

***Sporormia longipes*, sp. nov. (Fig. 1).**

Peritheciis sparsis plus minus superficialibus subglobosis glabris atris $\frac{1}{4}-\frac{1}{2}$ mm. diam., ostiolo brevi obtuso, contextu membranaceo parenchymatico e cellulis distinctis parvis (circiter 5μ) composito; ascis late clavatis vel elongato-clavatis in stipitem longissimum ($50-80\mu$) angustum saepe flexuosum attenuatis, octosporis, $120-145 \times 15-20\mu$; sporis asci apicem versus imbricato-3-stichis, cylindraceutis, utrinque rotundatis, rectis vel parum curvatis, quadrilocularibus, $26-33 \times 6-8\mu$, primum fuliginosis demum fuscis, facile secedentibus, articulis mediis minoribus subquadratis $5-6\mu$ longis, terminalibus longioribus oblongis ellipticisve.

Hab.—In fimo *Cariaci mexicanii* (Mexican Deer), *Capreoli capraeae* (Common Roe), *Camelopardalis giraffa* (Giraffe), *Caprae dorcadis* (Dorcas Goat), *Caprae sinaiticae* (Sinaitic Ibex), Kew, Dec.—Feb. 1901.

Inter species affines (*S. leporinam*, Niessl., *S. Notarisii*, Carestia) ascis longe stipitatis distinguenda.

The shape of the ascus varies in the present species from elongate-clavate to broadly clavate or (in outline) almost battledore-shape. The ascus is, however, always long stalked, and this character distinguishes *S. longipes* from the allied species of *Sporormia* with tetramerous spores. *S. corynespora*, Niessl., a species with octomerous spores, approaches the present species in its long-stalked asci. In *S. longipes* the spores remain steel-grey in colour for a long time, but finally become brown.

***S. ovina* (Desmaz.), Sacc. (Fig. 4).**

Hormospora ovina, Desmaz., in Ann. sci. nat., 3^e sér. (Bot.), xvi, 317 (1851); Pirota, Mon. Spororm., 161 (1878).

S. ovina, Sacc. Syll. Fung. ii, 127 (1883).

S. gigantea, Hans., in Vidensk. Meddel. 1876, p. 319, Tab. VI, f. 46 & 47 (1876-77); Speg. in Michel. i, 231 (1878); Niessl. in Oest. Bot. Zeit. xxviii, 123 (1878); Pirota, Mon. Spororm. 148 (1878); Sacc. Syll. Fung. ii, 127 (1883); Wint. in Rabenh. Krypt.-Fl. Deutschl., Bd. i, Abth. 2, 183 (1887).

Perithecia dark brown or black, scattered, glabrous, subimmersed, basal part subglobose narrowed upwards into a rather thick conical or subcylindrical neck; asci oblong or oblong-clavate, narrowed below into

a distinct stalk, 8-spored, $320-370 \times 50-55 \mu$, spores 2-4-seriate, $120-155 \times 17-20 \mu$, imbricate and arranged in subparallel rows, elongate-cylindrical, straight or (usually) slightly curved, tetramerous, deeply constricted and readily separating at the septa, median cells oblong or cylindric with square ends, terminal cells elongate-conoid, each spore surrounded by a rather broad layer of mucilage.

Hab.—On Rabbit-dung, Kew, Jan. 1901. (Distrib.—France, N. Italy, Denmark, Germany; on dung of Sheep and Cow.)

A comparison of Desmazière's '*Hormospora ovina*' in Desmaz. Crypt. Fr., 2^e sér., nr. 98, with authentic specimens (coll. Hansen) of *S. gigantea*, Hans., in the Kew Herbarium, shows the two plants to be identical, and as *ovina* is the older specific name, this must be used for the present *Sporormia*.

S. pulchella, Hans. (Fig. 2).

S. pulchella, Hans., in Vidensk. Meddel. 1876, p. 320, Tab. IX, ff. 23-25 (1876-77); Pirota, Mon. Spororm. 145 (1878); Niessl. in Oest. Bot. Zeit. xxviii, 95 (1878); Sacc. Syll. Fung. ii, 123 (1883); Wint. in Rabenh. Krypt.-Fl. Deutschl., Bd. i, Abth. 2, 181 (1887).

Perithecia gregarious or scattered, black, almost spherical, with a very short bluntly conical or papilliform neck, about $\frac{1}{4}$ mm. across, almost completely immersed; asci numerous, narrowly long-cylindrical, sometimes flexuose, rounded at the apex, narrowed below into a stalk, $110-150 \times 9-10 \mu$, 8-spored; paraphyses numerous, delicate, filiform, septate, sometimes branched, about as long as the asci; spores uniseriate, subcylindrical, straight or curved, $17-20 \times 5 \mu$, surrounded at first by a narrow layer of mucilage, tetramerous, constricted at the septa and readily separating, median cells somewhat barrel-shaped, terminal cells conical or subovate.

Hab.—On Horse-dung, Kew Green, England, Dec. 1900. (Distrib.—Denmark, Austria; on Cow- and Sheep-dung.)

S. pulchella is easily recognized by the numerous narrowly cylindrical asci with uniseriate spores.

S. fimetaria, De Not. (Fig. 19).

S. fimetaria, De Not., Micr. ital. dec. v, p. 10 (1849); Auersw. in Hedwigia, vii, 69, Tab. I, f. vi (1868); Pirota, Mon. Spororm. 160 (1878); Sacc. Syll. Fung. ii, 132 (1883); Wint. in Rabenh. Krypt.-Fl. Deutschl., Bd. i, Abth. 2, 187 (1887).

Hormospora fimetaria, De Not., in Giorn. Bot. Ital., tome ii, 47 (1884).

Sphaeria fimetaria, Rabenh. Herb. Myc., ed. 1, nr. 1733.

Perithecia densely gregarious, small, $140-175\ \mu$ in diam., subglobose with a minute papilliform ostium, wall membranous, parenchymatous, composed of rather distinct cells up to $10\ \mu$ wide; asci numerous, shortly cylindrical, usually slightly curved, $60-75 \times 13-15\ \mu$, narrowed below into a very short stalk, 8-spored, spores arranged parallel to each other, forming a single bundle in the ascus, narrowly long-cylindrical, $48-50 \times 4\ \mu$, 15-18-celled, segments readily separating, middle segments broader than long, $2.5\ \mu$ long, two end segments about $4.5\ \mu$ long.

Hab.—On Cow-dung, Aboyne, Scotland (Herb. Berk.). (Distrib.—Europe: Germany, Italy; N. America: U. S. A., South Carolina; on Cow-dung.)

S. fimetaria is one of the most distinct species of the genus, and is at once recognized by the parallel arrangement of the long, narrow, many-celled ascospores, which are collected into a single fascicle in the ascus. The fungus described under the name '*Sporormia fimetaria*, De Not.' by Schroeter in Cohn's Krypt.-Fl. Schles. is evidently, from the characters given, specifically distinct from the present plant. The examples of *S. fimetaria* from the United States (Aiken, S. C., Ravenel, nr. 2263) are in the Kew Herb., and agree exactly with the European plant.

S. minima, Auersw.; Sacc. Syll. Fung. ii, 124 (1883).

Hab.—On dung of Giraffe (*Camelopardalis giraffa*) and Dorcas Goat (*Capra dorcas*), Kew, Dec. 1900.

S. intermedia, Auersw.; Sacc. Syll. Fung. ii, 126 (1883).

Hab.—On dung of Giraffe (*Camelopardalis giraffa*), Dec. 1900; on Rabbit-dung, Reigate, Dec. 1900; on Horse-dung, Epping Forest, Oct. 1900.

S. megalospora, Auersw.; Sacc. Syll. Fung. ii, 126 (1883).

Hab.—On Horse-dung, Kew Green, Dec. 1900.

Sporormiella nigropurpurea, Ell. & Everh. (Fig. 3).

S. nigropurpurea, Ell. & Everh., N. Amer. Pyren., 136 (1892); Sacc. Syll. Fung. xi, 330 (1895).

Perithecia soft, carnosae, aggregated into groups and forming more or less extended continuous black patches; asci numerous, $110-140 \times 10-11\ \mu$, narrowly cylindrical, often flexuose, narrowed below into a stalk, 8-spored; paraphyses numerous, filiform, very slender; spores usually biseriate in the upper part of the ascus, subcylindrical, 18-

$20 \times 5-6 \mu$, surrounded at first by a narrow layer of mucilage, nearly straight, tetramerous, median cells subglobose, terminal cells subovate.

Hab.—On Cow-dung, Kew, Jan. 1901. (Distrib.—United States (New Jersey), on Cow-dung.)

The Kew specimens are identical with authentic examples (coll. Ellis) of *Sporormiella nigropurpurea* in the Kew Herbarium. The genus *Sporormiella* was founded by Ellis and Everhart (l. c.) with the following characters: 'Perithecia soft carnose, embedded in a flattish semi-immersed subcarnose stroma. Asci and sporidia as in *Sporormia*.' Only the single species *S. nigropurpurea* of the same authors is known, and in this the perithecia are described as 'buried in the soft flattish stroma, which is $\frac{1}{2}$ –1 cm. across, or by confluence more, slightly raised above the surface of the matrix, dark grey outside, and, like the perithecia, purplish black within.' A true stroma, however, such as is indicated by the above description, was not found either in Ellis' New Jersey specimens, mentioned above, nor in the Kew examples; in both cases the soft carnose perithecia being only aggregated into continuous patches. It must be noted that the presence of a definite stroma is the only character relied upon to separate the present genus from *Sporormia*. Moreover, except in the dense grouping of the perithecia, and in the ascospores being usually biseriate instead of uniseriate, both the Kew and Ellis' specimens agree so closely in all characters with *Sporormia pulchella*, Hans., that even the specific distinctness of the present plant seems almost doubtful.

Microascus variabilis, sp. nov. (Fig. 24).

Peritheciis gregariis matrice subimmersis parvulis $150-200 \mu$ diam., subglobosis, ostiolo minuto papilliformi vel plus minus elongato cylindraceo interdum flexuoso, nigris, subcarbonaceis, fragilibus, contextu parenchymatico e cellulis densis opacis distinctis minutis $3-5 \mu$ latis composito, peritheciis parte superiore pilis rigidis paucis sparsis fuliginosis interdum obsoletis instructa, ascis minutis subglobosis subgelatinosis $7-8 \mu$ diam., citissime diffluentibus octosporis; sporis crasse (interdum irregulariter) lunulatis, minutis, $3-3.5 \mu$ longis, utrinque obtusis, levibus, dilutissime brunneis.

Hab.—In fimo *Dolichotidis patagonicae* (Patagonian Cavy), Kew, Jan. 1901.

The above species seems to be allied to *M. longirostris*, Zukal, but differs in the shorter or even obsolete neck of the perithecium, the much smaller asci, and the smaller spores, with obtusely rounded, not

acute, ends. The spores of *M. variabilis*, seen in the mass, have a faint brownish tinge; we were not able to observe any layer of mucilage surrounding each spore, as Zukal has recorded in the case of *M. longirostris*. The asci are extremely evanescent, and difficult to see—the perithecium containing as a rule only free spores. The latter are usually broadly lunulate in outline, and somewhat flattened; sometimes, however, they are slightly irregular in shape. The variability in the shape of the perithecium is very marked—the ostiolum being sometimes papilliform or even hardly visible, at others well-defined and forming a distinct more or less cylindrical neck, which is sometimes flexuous.

M. nidicola, sp. nov. (Figs. 62–65).

Peritheciis nigris glabris carbonaceo-membranaceis sparsis matrice subimmersis subglobosis $\frac{1}{3}$ mm. diam. ostiolo brevi conico, contextu parenchymatico densissimo e cellulis polygonis nigro-fuscis plus minus opacis $8-10\mu$ latis composito; ascis numerosis subgelatinosis ellipticis vel globoso-ellipticis $10-13 \times 6-8\mu$ octosporis citissime diffluentibus; sporis minutis anguste sublunulatis utrinque acutis laevibus hyalinis vel stramineis $7.5-8 \times 2\mu$.

Hab.—In nido vetusto *Bombi* sp., sociis *Myxotricho setoso* (Eidam), Schroet., et *Arachnioto candido* (Eidam), Schroet., Kew, Mar. 1901.

M. longirostri, Zukal, sporis lunulatis comparanda, sed peritheci forma et glabritate, ascis minutis nec non sporis angustioribus majoribus longe recedens.

The above species occurred sparingly on an old nest of a Wild-bee (*Bombus* sp.). The nest, which was dug up in the Royal Gardens, Kew, was covered on the surface with large patches of *Myxotrichum setosum* and *Arachniotus candidus*. The wall of the perithecium, unlike that of *M. variabilis*, is very dense and opaque, so that its cellular structure is not readily apparent. The asci are subgelatinous, and very quickly dissolve in water, setting free the narrow somewhat lunate-shaped spores. The spores become finally of a pale straw colour, and the ascus itself is sometimes tinged with the same colour.

Spumatoria, gen. nov.

Perithecia subglobosa, semi-immersa, demum superficialia, membranacea, in rostrum longum cylindraceum apice fimbriatum plus minus dilatatum attenuata, ascis tenuibus, evanescentibus, octosporis, sporis didymis, hyalinis, demum in spuma mucilaginosa ex ore rostri ejectis; paraphysibus indistinctis.

***S. longicollis*, sp. nov. (Fig. 27).**

Peritheciis sparsis 0.75-1 mm. altis semi-immersis demum superficialibus subglobosis olivaceis membranaceis contextu parenchymatico e cellulis polygonis 6-10 μ latis composito glabris vel basi hyphis repentibus instructis, in rostrum longissimum cylindricum atrum rugulosum apice fimbriatum plus minus dilatatum abrupte attenuatis; ascis cylindraceis, 110-130 \times 13-15 μ deorsum in stipitem attenuatis, octosporis, citissime diffluentibus; sporis monostichis oblongis utrinque rotundatis 15-19 \times 5 μ hyalinis 1-septatis medio haud constrictis demum in spuma mucilaginoso ex ore rostri ejectis; conidiis in eodem perithecio productis, oblongis, hyalinis, 17-20 μ longis, 1-septatis, basi plus minus attenuatis, in conidiophoris simplicibus brevibus acrogenis.

Hab.—In fimo equino, Epping Forest, Essex, October, 1900.

The perithecia of the present fungus, which appeared on some Horse-dung soon after it was collected, readily attract attention by reason of the long upright black cylindrical beaks. At maturity the spores are seen issuing forth from the apex of the beak in the mucilage formed by the deliquescence of the asci, &c. The mouth of the beak is composed of loosely arranged easily separating hyphae, which during the ejection of the spores are forced apart by the mucilaginous mass, so that the apex of the beak at this time is more or less dilated.

The conidia precede the asci in the same perithecium, and the Fungus in this stage appears to be identical with *Rhyncophoma*, Karst. emend. (see Allescher, in Rab. Krypt.-Fl. Deutschl., 6. Abth., vol. i, p. 711).

***Xylaria pedunculata* (Dicks.), Fr.; Sacc. Syll. Fung. i, 332 (1882).**

Sphaeria pedunculata, Dicks., Pl. Crypt. Brit. iv, 27, Tab. XII, f. 8 (1801).

The spores in Dickson's type-specimen measure 50 \times 22-24 μ , and so slightly exceed the measurement ('40 \times 20') given by Saccardo, &c.

Hypocraeaceae. *Sphaeroderma fimbriatum*, Rostr. (Fig. 23).

S. fimbriatum, Rostr., in Meddel. om Groenland, xviii, 67 (1895); Sacc. Syll. Fung. xi, 356 (1895).

Perithecia scattered or subgregarious, superficial, about $\frac{1}{3}$ mm. diam., globose with a mamilliform ostiolum, which is surmounted by a tuft of short (60-100 \times 4-6 μ) rigid colourless hairs, otherwise glabrous or with a few spreading hyphal outgrowths near the base, at first yellowish,

becoming dull red, wall thin, soft, membranous, composed of rather large ($10-20\mu$ wide) irregularly shaped cells; asci obovate or subpyriform, $80-100 \times 22-25\mu$, rounded at the apex, narrowed below into an evident stalk, 8-spored; spores inaequilateral, somewhat lemon-shaped, $21-25 \times 10-11\mu$, fuliginous, opaque.

Hab.—On the dung of Guinea Pig, Kew; Jan. 1901. (Distrib.—E. Greenland, on dung of Reindeer.)

The above species occurred associated with *Pleuroascus Nicholsoni* (see p. 330). The hairs, forming a tuft round the ostium, are often somewhat swollen at their base (Fig. 23). We have not seen an authentic specimen of Rostrup's species, but the author's description, given below, seems to point to the present plant: 'Perithecia minima, sphaeroidea, rubella, ostiolo fimbriato, subiculo obsoleto; asci cylindraceo-clavati, longit. $100-110\mu$, crass. 20μ ; sporae distichae, initio hyalinae, dein fuscae, ellipsoideae, long. 20μ , crass. $11-12\mu$.'

S. Hulseboschii, Oudem. (Figs. 57-61).

S. Hulseboschii, Oudem., in Nederl. Kruid-Archief, ser. 2, iv. 523 (1886); Sacc. Syll. Fung. ix, 949 (1891).

Perithecia scattered or subgregarious, superficial, $\frac{1}{4}-\frac{1}{2}$ mm. in diam., yellowish or with a reddish tinge, subglobose with a minute ostium, which is surrounded by short (about 30μ long) colourless hairs, wall of perithecium soft, thin, composed of rather large, irregularly shaped cells, about $15-20\mu$ wide; asci pyriform, $50-60 \times 25\mu$, 8-spored, evanescent; spores arranged in two or three rows towards the apex of the ascus, lemon-shaped, often inaequilateral, minutely truncate at each end, $18-20 \times 9-10\mu$, at first colourless, then pale olivaceous, becoming finally fuliginous and opaque, the tip at each end of spore remaining pallid for some time.

Hab.—On Rabbit-dung, Leith Hill, Surrey, England, Mar. 1901. (Distrib.—Netherlands, on Rabbit-dung.)

The spores of the present species are lemon-shaped, and minutely truncate at each end. As is noted in Oudemans's original description, the extreme ends of the spore commonly remain subhyaline for some time after the rest of the spore has become dark-coloured.

Melanospora discospora, sp. nov. (Figs. 36-38).

Peritheciis sparsis rare subgregariis minutis $110-130\mu$ diam. $180-200\mu$ altis subimmersis glabris subglobosis flavicantibus membranae contextu parenchymatico molli tenui e cellulis irregularibus circiter 10μ latis composito rostro breve-cylindraceo recto obtuso $60-80\mu$

longo apicem versus subhyalino; ascis oblongis circ. $35 \times 14 \mu$, octo-sporis citissime diffluentibus sporis discoideis a fronte orbicularibus $7-9 \mu$ diam., a latere ellipticis 4μ latis atrobrunneis, ad rostri apicem singulatim eructatis et tandem massam subglobosam nigrum formantibus.

Hab.—In fimo *Caprae sinaiticae* (Sinaitic Ibex) et *Ovis burrhel* (Burrhel Wild Sheep), Kew, Mar. 1891.

A congeneribus sporis discoideis statim dignoscenda.

The minute perithecia, when perfectly ripe, attract attention from the black globular mass of spores at the apex of the beak. Each spore is singly expelled from the basal part of the perithecium up the neck, and in the process of being discharged is clearly visible in the nearly hyaline upper part of the beak. The spores are very dark brown, and perfectly discoid in shape.

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EXPLANATION OF FIGURES IN PLATES XVII AND XVIII.

Illustrating Messrs. Massee and Salmon's paper on Coprophilous Fungi.

Fig. 1. *Sporormia longipes*, sp. nov.; perithecium, $\times 45$; cells of outer wall of same, $\times 400$; two asci and spores, $\times 400$.

Fig. 2. *S. pulchella*, Hans.; perithecium, $\times 64$; cells of outer wall of same, $\times 400$; two asci and spores, $\times 400$.

Fig. 3. *Sporormiella nigropurpurea*, Ell. and Everh.; group of three perithecia, $\times 70$; two asci, paraphyses, and spores, $\times 400$.

Fig. 4. *Sporormia ovina* (Desmaz.), Sacc.; perithecium, $\times 80$; two spores, $\times 400$.

Fig. 5. *Sordaria Winteri*, Karst.; perithecium, $\times 40$; spore, $\times 400$.

Fig. 6. *S. anserina* (Rabenh.), Wint.; perithecium, $\times 25$; spore, $\times 400$.

- Fig. 7. *S. hirta*, Hans.; perithecium, $\times 25$; spore, $\times 400$.
 Fig. 8. *S. coprophila*, Ces. and De Not.; four stages in the development of the spore, $\times 400$ (see p. 315).
 Fig. 9. *S. macrospora*, Auersw.; perithecium, $\times 25$; apex of ascus and two spores, $\times 400$.
 Fig. 10. *S. fimicola* (Rob.), Ces. and De Not.; spore, $\times 400$.
 Fig. 11. *S. minima*, Sacc. and Speg.; perithecium, $\times 200$; ascus and two spores, $\times 400$.
 Fig. 12. *S. curvicolla*, Wint.; perithecium, $\times 25$; two spores, $\times 400$.
 Fig. 13. *S. setosa*, Wint.; perithecium, $\times 25$; ascus, $\times 255$; spores, $\times 400$.
 Fig. 14. *S. pleiospora*, Wint.; perithecium, $\times 25$; two spores, $\times 400$.
 Fig. 15. *S. neglecta*, Hans.; perithecium, $\times 25$; spore, $\times 400$.
 Fig. 16. *S. fimiseda*, Ces. and De Not., var. *appendiculata* (Auersw.); perithecium, $\times 25$; three spores, (a) young stage, $\times 400$.
 Fig. 17. *Sordaria*, sp. (see p. 341); perithecium, $\times 25$; single cell of outer wall of same, $\times 400$.
 Fig. 18. *Delitschia moravica*, Niessl.; perithecium, $\times 80$; two spores, $\times 400$; single spore, $\times 670$.
 Fig. 19. *Sporormia fimetaria*, De Not.; perithecium, $\times 80$; cells of outer wall of same, $\times 400$; ascus and spores, $\times 400$.
 Fig. 20. *Delitschia insignis*, Mout.; perithecium, $\times 10$ and 30 ; spores, $\times 400$.
 Fig. 21. *Sordaria globosa*, sp. nov.; perithecium, $\times 25$; spore, $\times 400$.
 Fig. 22. *Delitschia Winteri*, Plowr.; perithecium, $\times 25$; spore, $\times 400$.
 Fig. 23. *Sphaeroderma fimbriatum*, Rostr.; perithecium, $\times 40$; cells of outer wall of same, $\times 400$; ascus and spores, $\times 400$; to right, two hairs surrounding the ostiolum, $\times 400$.
 Fig. 24. *Microascus variabilis*, sp. nov.; two perithecia, $\times 95$; cells of outer wall of perithecium and seta-like hair from same, $\times 400$; spores, $\times 400$; ascus and spores, $\times 670$; single spore, $\times 1000$.
 Fig. 25. *Pleuroascus Nicholsoni*, gen. nov. sp. nov.; perithecium separated from the subiculum, and showing the spiral appendages attached to it, $\times 80$; cells of outer wall of same, $\times 400$; spiral appendage, $\times 400$; ascus and spores (a) $\times 400$, (b) $\times 670$, (c) $\times 1000$.
 Fig. 26. *Magnusia Bartlettii*, sp. nov.; perithecium, $\times 45$; cells of outer wall of same, $\times 400$; asci and spores, $\times 400$.
 Fig. 27. *Spumatoria longicollis*, gen. nov. sp. nov.; perithecium ejecting spores, $\times 90$; hyphae surrounding ostiolum, $\times 400$; cells of outer wall of basal part of perithecium, $\times 400$; ascus and spores, $\times 400$; to left of perithecium five stages in the development of the conidia (*Rhyncophoma* stage), $\times 400$.
 Fig. 28. *Eurotium microsporum*, sp. nov.; perithecium, $\times 45$; cells of outer wall of same, $\times 400$; two spores, $\times 980$; group of asci, $\times 1000$.
 Fig. 29. *E. insigne*, Wint.; perithecium, $\times 25$; to left, cells of outer wall of same, $\times 400$; ascus and spores, $\times 400$; single spore, $\times 670$.
 Figs. 30-32. *Myxotrichum uncinatum* (Eidam), Schroet.; Fig. 30, single tuft growing on Rabbit-dung, $\times 52$; three spores from same, $\times 400$; ditto and ascus, $\times 670$; Figs. 31, 32, tuft growing on the dung of Patagonian Cavy, $\times 45$; spores from same, $\times 400$ and 670 ; Fig. 32, appendage proceeding from the vegetative mycelium, $\times 400$.
 Figs. 33-34. *Rhyparobius ascophanoides*, Sacc.; Fig. 33, apothecium, seen from

above, and in section, $\times 45$; Fig. 34, ascus containing nearly ripe spores, and paraphyses, and two ripe spores, $\times 400$.

Fig. 35. *Endomyces coprophilus*, sp. nov., part of creeping mycelium with three ripe asci and one immature ascus, $\times 400$; ascus with four and eight spores, $\times 1000$.

Figs. 36-38. *Melanospora discospora*, sp. nov.; Fig. 36, perithecium ejecting spores, $\times 95$; Fig. 37, young ascus, $\times 400$; Fig. 38, ripe spores, $\times 400$.

Figs. 39-40. *Eurotium insigne*, Wint.; conidial stage (= *Gliocladium penicilloides*, Corda); Fig. 39, single conidiophore, $\times 95$; Fig. 40, apex of same, and conidia, $\times 400$.

Figs. 41-44. *Thelebolus stercoreus*, Zukal; Fig. 41, apothecium, with single protruding ascus, $\times 125$; Fig. 42, ascus, showing mode of dehiscence (magnified); Figs. 43, 44, apex of same, more highly magnified.

Fig. 45. '*Ascozonus oligoascus*, Heimerl' (see p. 327); apothecium, $\times 255$, and ascus, $\times 400$.

Figs. 46, 47. *Ryparobius*, sp. on Goose-dung, Kew (see p. 320); apothecium, $\times 200$; two asci from the same apothecium, $\times 400$.

Figs. 48-51. *Saccobolus quadrisporus*, sp. nov.; Fig. 48, apothecium, $\times 52$; Fig. 49, ascus, $\times 400$; Fig. 50, group of spores, discharged from the ascus, but still surrounded by the mucilaginous 'sac,' $\times 400$; Fig. 51, spores, $\times 670$.

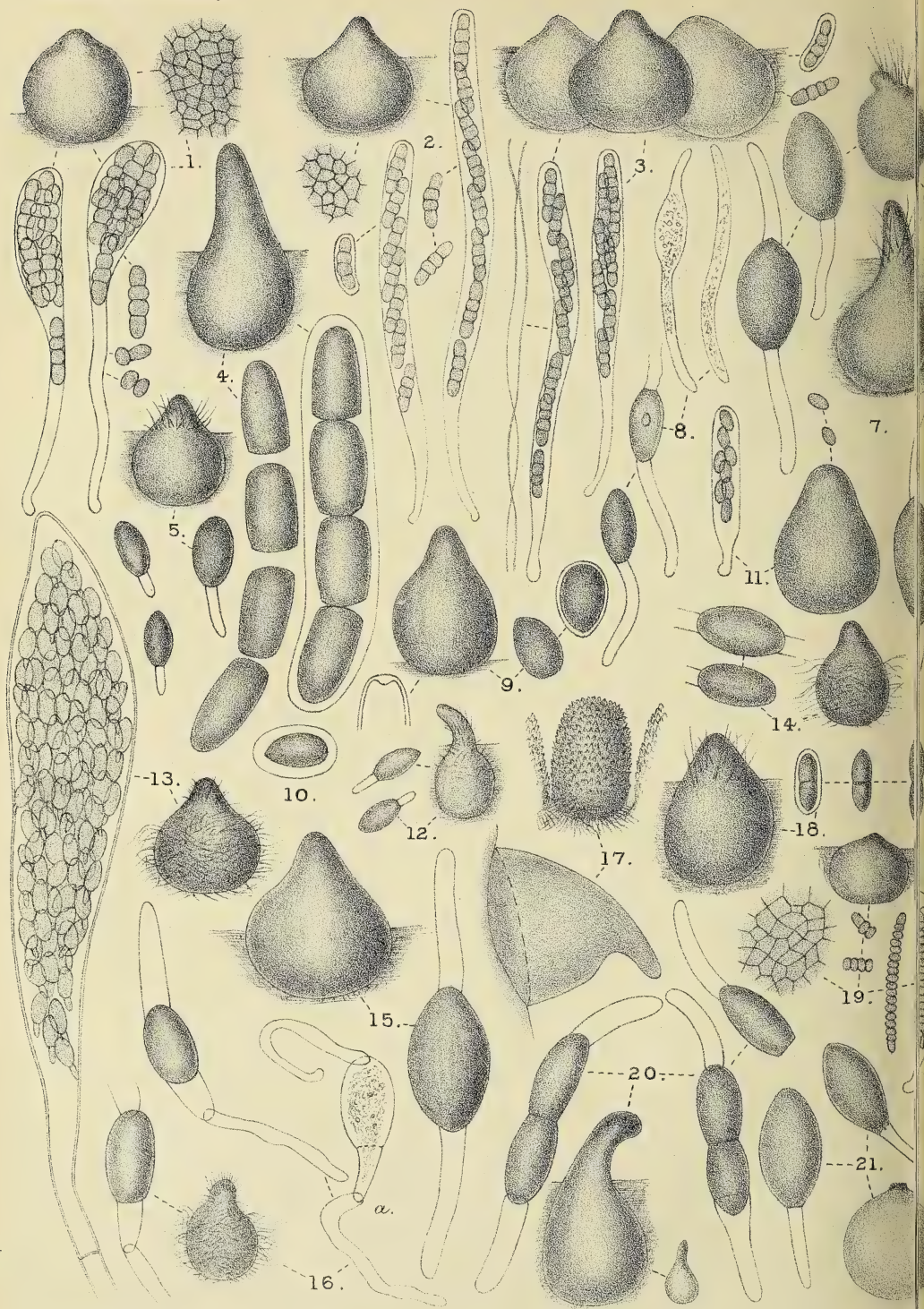
Figs. 52-55. *Ascobolus perplexans*, sp. nov.; Fig. 52, ascophore, $\times 25$; Fig. 53, ascus and paraphyses, $\times 400$; Fig. 54, spores, $\times 400$; Fig. 55, single spore, $\times 1000$.

Fig. 56. Two spores of *Ascobolus glaber* (*A. albidus*, Crouan), taken direct from apothecia growing on Rabbit-dung, and germinating in a hanging-drop of dung-decoction, $\times 400$ (see p. 317).

Figs. 57-61. *Sphaeroderma Hulseboschii*, Oudem.; Fig. 57, perithecium, $\times 52$; Fig. 58, cells of outer wall of same, $\times 400$; Fig. 59, ostium of perithecium, $\times 400$; Fig. 60, ascus and spores, $\times 400$; Fig. 61, immature spore, $\times 400$.

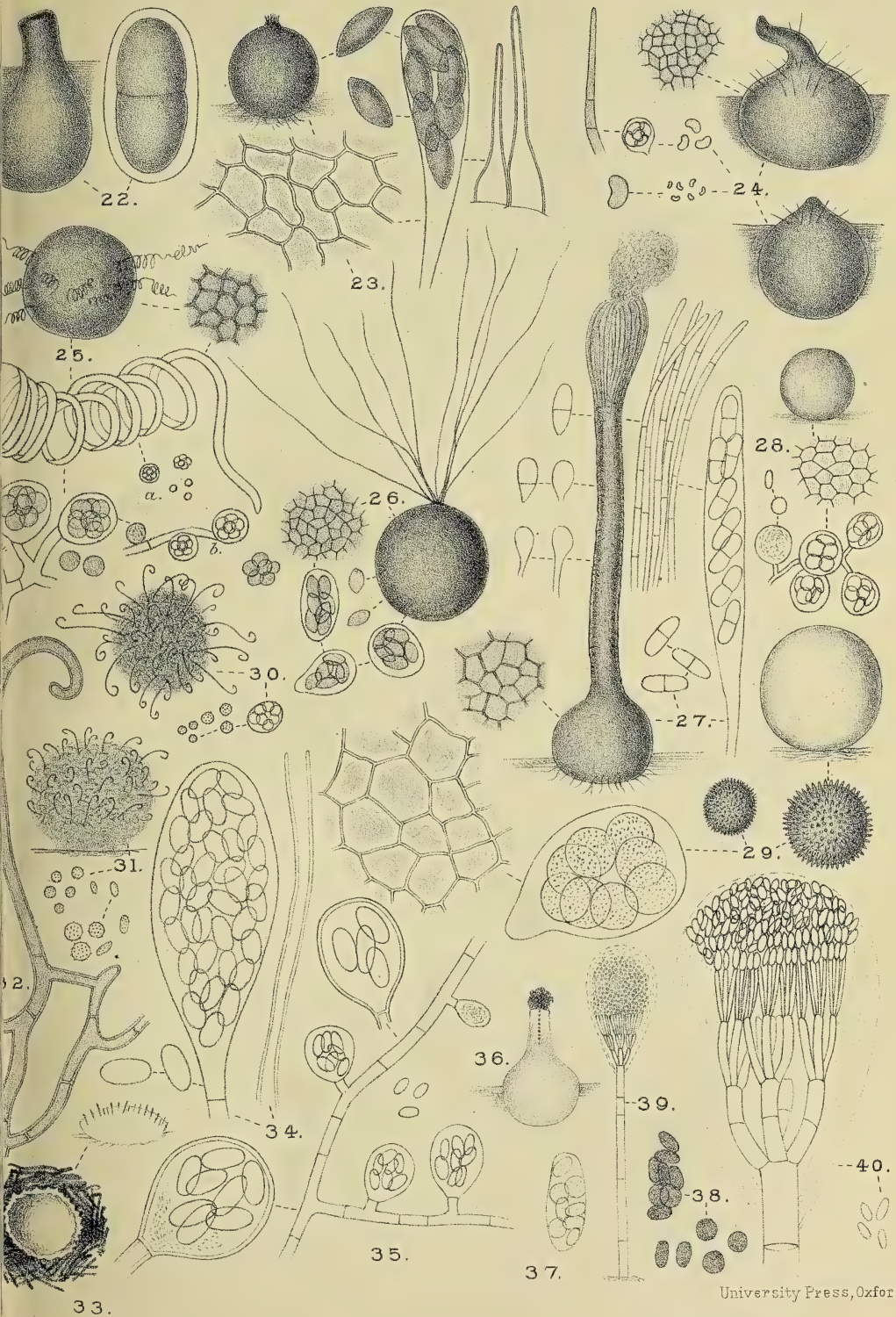
Figs. 62-65. *Microascus nidicola*, sp. nov.; Fig. 62, perithecium, $\times 95$; Fig. 63, cells of outer wall of same, $\times 400$; Fig. 64, asci and spores, $\times 400$; Fig. 65, ditto, $\times 670$.

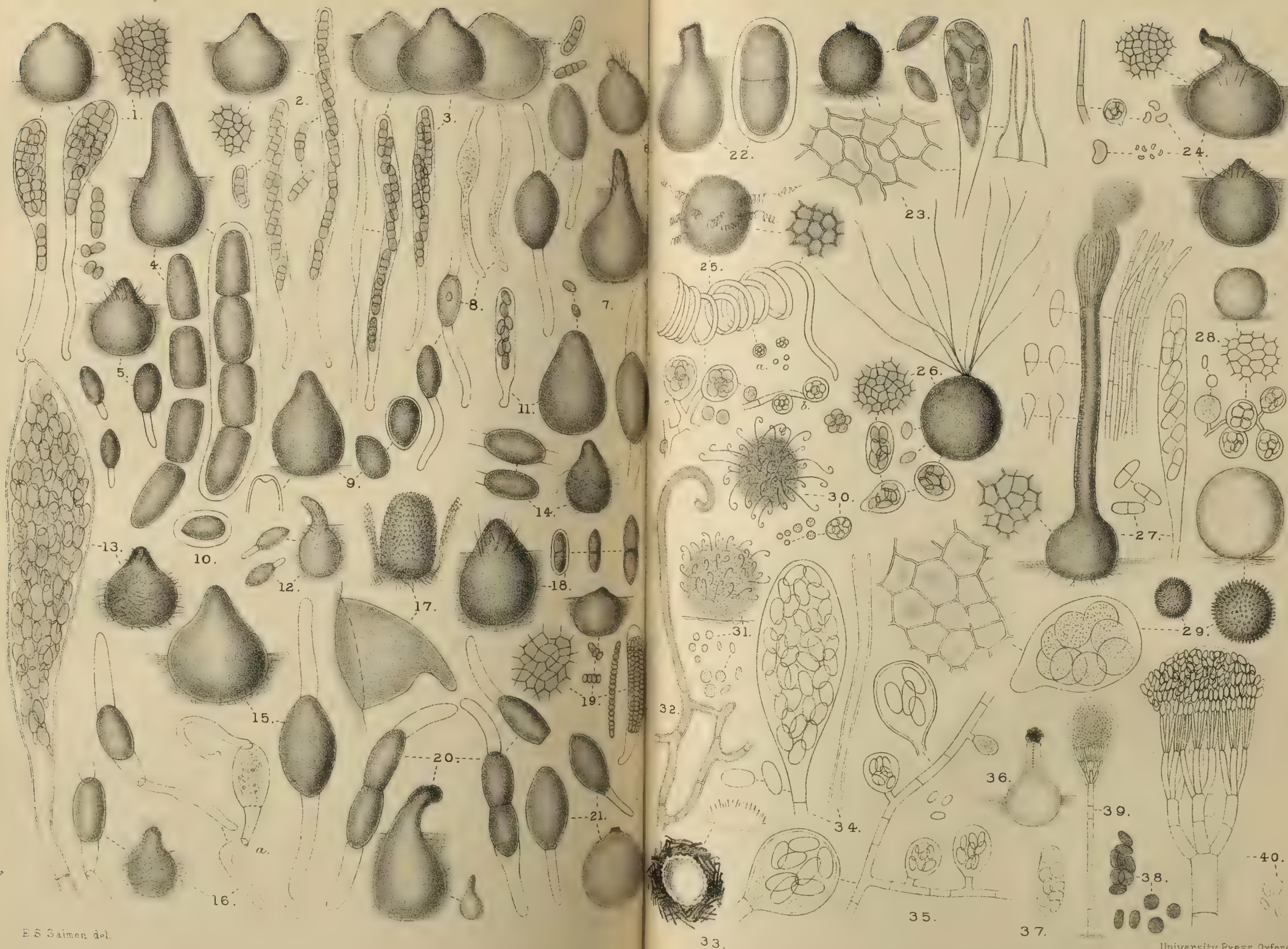
Fig. 66. *Thelebolus stercoreus*, Zukal; transverse section through an apothecium containing two asci, $\times 400$.



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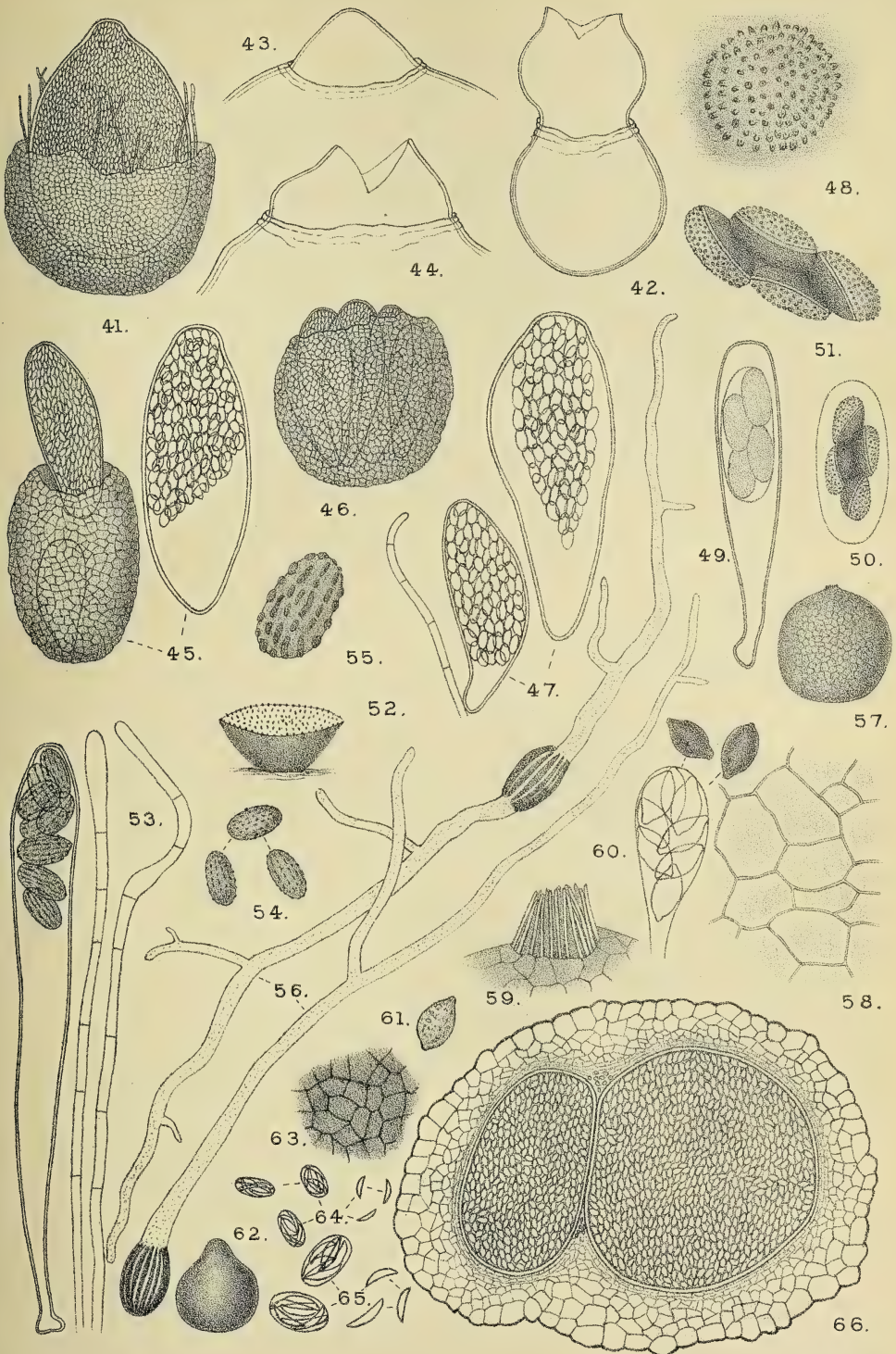




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Comparative Anatomy of the Hymenophyllaceae, Schizaeaceae and Gleicheniaceae.

II. On the Anatomy of the Schizaeaceae¹.

BY

L. A. BOODLE, F.L.S.

—♦—
With Plates XIX, XX, and XXI.
—♦—

THE Anatomy of the Hymenophyllaceae formed the subject of the first part of this paper, which was published last year². The present part is concerned with the anatomy of the Schizaeaceae. A monograph of this Order, which contained a large amount of valuable information regarding the anatomy, was published by Prantl ('81). As many of the observations to be described below are confirmatory of Prantl's statements, his work will be referred to at the end of the paper, so as to avoid too numerous references.

Of the anatomical points to be dealt with here, the structure of the vascular tissue of the stem and petiole occupy the chief place, and form in some respects a more interesting study than in the Hymenophyllaceae, on account of the very diverse types found within the Order. The simply constructed stele of *Lygodium* is not far removed in type from certain species of

¹ From the Jodrell Laboratory, Royal Gardens, Kew.

² Annals of Botany, vol. xiv, p. 455.

the Hymenophyllaceae, but the dialystelic stem of *Anemia Phyllitidis* has no counterpart in that Order. Seedling plants of both these types were examined, in order to trace the manner in which the structure of the mature stem is attained in the ontogeny of the plant, and if possible to draw some conclusion as to the homologies of the mature structure. Especially in the case of *Anemia* and *Schizaea* several anatomical points have been left for further investigation, and as new material of some species has recently been obtained, it is intended to publish some additional observations at a future date.

In the Schizaeaceae Hooker and Baker ('74) include five genera: *Schizaea*, Sm., *Anemia*, Sw., *Mohria*, Sw., *Trochopteris*, Gardn., and *Lygodium*, Sw. *Trochopteris*, which is represented by a single species, is placed by Prantl ('81) as a subgenus of *Anemia*¹, thus reducing the genera to four. Material of *Trochopteris* was not obtained, but the structure was investigated in *Lygodium*, *Schizaea*, *Anemia*, and *Mohria*, and the results will now be described, and will be arranged under each genus taken in the order just given.

Some further observations on the sieve-tubes and bundle-fibres of the Hymenophyllaceae are included towards the end of the paper, where a more general treatment of certain tissues, &c. is given.

LYGODIUM, habit.

The stem of *Lygodium* is a horizontal rhizome often much branched at short intervals, and apparently dichotomously, but with some of the branches occasionally arrested. The roots are borne on the lower surface, without marked regularity, but approaching distichous arrangement in places. The leaves are borne at short intervals on the upper surface of the rhizome, and according to Prantl ('81, p. 2) form a single row. This is often approximately correct, but especially where the leaves are rather crowded, they tend to distichous

¹ Prantl and other authors write the generic name *Anemia*, in order to make its Greek derivation correct.

arrangement. The petiole is upright and is continued into the climbing rachis which has indefinite growth, and bears pinnae at intervals. The pinna is usually quite short and stalk-like and divides dichotomously to produce secondary pinnae, but frequently at its apical region, i.e. at the fork, a bud-like body is produced, which afterwards is circinate, and then lengthens to produce a secondary rachis. The secondary pinnae may be simple or may be further divided dichotomously or pinnately.

In all the genera of the Order the form of the leaves, venation, fructification, &c. are fully described in Prantl's monograph.

LYGODIUM, stem.

The rhizome of *Lygodium dichotomum*, Sw., has a thick cortex, in which three zones may be distinguished. The outer cells are thin-walled and brown, and graduate into the thick-walled sclerotic elements of the middle part of the cortex. The latter cells have dark reddish-brown walls with numerous pits and distinct stratification. A similar brown colouring matter present in the walls is familiar in the sclerotic tissue of most Ferns and has been shown by Walter ('90, p. 18) to be phlobaphene, which probably belongs to the group of humic substances. This colouring matter is slowly removed from the sclerotic tissue of *Lygodium* by the action of Eau de Javelle, and the decolourized walls then give the cellulose reaction with Schulze's solution. This agrees with what Poirault ('93, p. 127) found in certain other Ferns. Succeeding the sclerotic tissue abruptly is the third or innermost zone of the cortex, which consists of from three to five layers of rather thin-walled cells, whose walls are suberized. Many of these cells are slightly rounded off at the corners, when seen in transverse section. In a tangential section they are from two to five times as long as broad, and often show a chain of small oval intercellular spaces between their vertical walls, similar to those seen among the palisade-cells of some Angiospermous leaves. The intercellular spaces thus run in the

radial direction, and their function is probably connected with the aeration of the tissues of the stele. In Fig. 1, Plate XIX, the layer of suberized cells may be recognized by comparison with the high-power drawing, Fig. 2, where this tissue (*s.l.*) is seen to lie between the sclerotic middle cortex, one layer of which is shown at the top of the drawing, and the endodermis (*e.*). The endodermis is easy to distinguish; in young stems it shows the usual suberized bands in its cells, and in old stems all its cell-walls are suberized. Its cells become yellow or brownish. The pericycle is mostly from three to four cells thick. Its cells, at an early stage, show radial seriation with their radial walls corresponding with those of the endodermal cells (as seen to some extent in Fig. 3). This arrangement is, however, upset by subsequent displacement (Fig. 2). The protophloem forms a ring, broken here and there, and consists of rather small sieve-tubes, often flattened in old stems, and easily distinguished from the succeeding band of metaphloem, which is composed of large sieve-tubes and phloem-parenchyma. The walls of the sieve-tubes are rather thick, and stain strongly with haematoxyline (Fig. 2, *ph.*). In thick unstained transverse sections the sieve-tubes are seen to differ by their scanty contents from the phloem-parenchyma.

In longitudinal section the sieve-tubes, which are much elongated elements with oblique end-walls, appear empty except for the presence of numerous granules on the walls. Usually, at any rate, no nuclei are present. This character readily distinguishes the sieve-tubes from the pericyclic cells, which have evident protoplasm and nuclei. The walls of the sieve-tubes stain blue with Schulze's solution, often rather pale, but in some sections of the petiole they took an extremely deep-blue colour; in this case the conjunctive parenchyma-cells in the phloem and xylem were also deeply stained, but the pericyclic cells were scarcely coloured. The sieve-plates remain nearly unstained by Schulze's solution, and the granules turn slightly yellow. The latter are often nearly restricted to the sieve-plates, and probably consist of proteid.

Part of a small sieve-tube from the rhizome of *L. dichotomum* is shown in Fig. 7. In the largest sieve-tubes the sieve-plates on the vertical walls are often much more crowded than in the specimen figured; the thick parts of the wall, in consequence, have a reticulate arrangement. Sieve-plates in section and surface view are easily seen in unstained longitudinal sections. In structure the sieve-tubes of *Lygodium* agree with those of other Ferns, but callus, as will be described below, appears to be absent.

A layer of conjunctive parenchyma (1-2 cells thick) separates the phloem from the xylem. The xylem occupies the centre of the stele, and forms a solid rounded mass about $\frac{3}{4}$ mm. to $2\frac{1}{2}$ mm. in diameter (Fig. 1). It consists of tracheides and parenchyma, the latter occurring chiefly as tortuous chains of cells, which are mostly uniseriate (Fig. 2). Short rows of cells and isolated cells (as seen in transverse section) also occur, but it is probable that the parenchyma really forms a connected system. The smallest tracheides, which are also the first to differentiate, and must be described as protoxylem, are scattered round the periphery of the xylem, and are not arranged in definite groups; see Fig. 2, and also the young stem Fig. 3, where the protoxylem-elements are the only tracheides differentiated. Especially in large steles in this species the protoxylem-elements may be much more numerous on the lower than on the upper side of the xylem, and may differentiate earlier on the lower side. The first-formed tracheides differ from typical protoxylem in not being annular or spiral. They are finely scalariform. This character no doubt implies that the formation of the protoxylem is subsequent to the elongation of the tissues. The tracheides of the remainder of the xylem are scalariform, and their order of differentiation is not regularly centripetal, but a number of single tracheides scattered throughout the xylem become fully developed, while the intervening ones are still thin-walled. The latter become subsequently thickened and the mature structure is attained. The intermediate stage of development with internal scattered tracheides is described by Russow ('72,

p. 97); and Prantl ('81, p. 27) evidently referred to this stage when he described the protoxylem as 'regellos über den Strangquerschnitt zerstreut.'

Many of the conjunctive-parenchyma cells in the xylem and also those bounding its periphery contain irregular refractive bodies, which prove to be silica-nodules. Several are shown in Fig. 2; the one marked *sz.* is adjacent to the nucleus of the cell. They show varied shape and texture, but a frequent type has a rod-like general outline, either blunt or pointed at both ends, the texture being sometimes loose and granular, but more often dense and solid-looking, with a botryoidal or mammillated surface. They occur singly in a large proportion of the cells mentioned above. The silica-nodule, when large, lies with its length along the length of the cell which contains it, and appears to be free in the cell cavity, but is possibly imbedded in a mucilaginous substance. In a piece of old rhizome the silica-nodules were specially well-developed, and many attained a length of $\frac{1}{16}$ mm. They can be isolated by boiling a piece of the stele in Schulze's macerating fluid till the tissues are completely dissolved, when the sediment consists entirely of silica-nodules. Fig. 5 is a drawing of a nodule isolated in this way. They are very distinct when mounted in methylated spirit or water, but almost unnoticeable in strong glycerine or sulphuric acid. This of course depends on the refractive index of the liquid. Decisive chemical tests were not employed, but it is easily shown, by rubbing them between a watch-glass and a glass-slide, that they are hard enough to scratch glass, hence it may be taken for granted that they consist of silica. The siliceous bodies were found to be about equally numerous and well developed in the rhizome of a plant grown at Kew and of one from Ceylon. Poirault ('93, p. 241) has described siliceous bodies of similar appearance in certain epidermal cells of the petiole in some species of Marattiaceae.

The structure of the node will be described later. The rhizome of *Lygodium* has a uniform type of structure in the species examined, and as it has been rather fully described in

L. dichotomum, it will only be necessary to mention a few differences of detail, observed in other species. The stele of *L. pinnatifidum*, Sw., is similar to that of *L. dichotomum*, but smaller. Silica-nodules are present, but in the specimen examined were less numerous. The walls of the suberized cells of the inner cortex become brown in the old rhizome. In *L. japonicum*, Sw., the protoxylem of the rhizome was seen to be similar to that of *L. dichotomum*. No siliceous nodules were observed. In *L. scandens*, Sw., the small scalariform tracheides are at the periphery of the xylem as in the other species, but they are less numerous and less regularly distributed than in *L. dichotomum*. The layer of suberized cells is several cells thicker than in that species, and much more lacunar in transverse section, adjacent cells being often completely rounded off from one another with the exception of a narrow neck-like prolongation connecting them and divided by a cross-wall at the middle point. Chains of intercellular spaces between the cells are seen in a tangential section as in the other species. In *L. palmatum*, Sw., the inner cortex instead of being represented by lacunar suberized cells, consists of sclerotic cells, which showed no intercellular spaces. In *L. reticulatum*, Schk., the tracheides of the rhizome are specially thick-walled, and no layer of suberized cells was observed. In *L. volubile*, Sw., the rhizome is of the usual type; the layer of suberized cells is yellowish and fairly lacunar.

The branching of the stem in *Lygodium* appears to be dichotomous, but no stage was met with, sufficiently early to prove whether it is a case of true dichotomy. The stele elongates so as to become oval, then becomes constricted into hour-glass form, and divides into two about equal-sized steles. No definite relation between the branching and the leaf-insertion could be made out, but in one or two cases a leaf-trace was attached to the stele just where it forked, in a median position, and the tracheides of the leaf-trace were attached to tracheides supplying the xylem of both branches. The apex of the rhizome, examined in the seedling of

L. japonicum, agreed with what Bower ('89, p. 322) found in *L. scandens*, i. e. it had a three-sided apical cell, and a comparatively flat apical cone.

LYGODIUM, petiole and node.

A transverse section of the petiole of *L. japonicum* is represented in Fig. 4. The xylem, which forms a solid mass at the centre of the bundle, has the same characters as in the rhizome, that is, it consists chiefly of large scalariform tracheides and chains of parenchymatous cells. It has three prominences, one median and two lateral, where the first formed tracheides occur. On the morphologically lower side (the upper side in Fig. 4) the prominence bears a pair of protoxylem-groups composed of spiral tracheides. Each of the two remaining prominences has one or two spiral tracheides connected with it, but most of the small peripheral tracheides in this region are scalariform. The protophloem forms a scarcely broken ring round the xylem, and the metaphloem attains its greatest thickness and includes its largest sieve-tubes in the three bays alternating with the three prominences of the xylem; the lateral bays being here considerably deeper than the median one. The pericycle is mostly from one to two cells thick. The endodermis is small-celled, and its cells contain a mucilaginous substance. The form of the xylem in transverse section varies according to the distance from the base of the petiole. Close to the base the xylem becomes much more rounded, while higher up in the petiole the prominences of the xylem become more and more pronounced. Some details on this point are given below under *L. dichotomum*. One or two granular bodies, probably siliceous, were observed in the petiole of *L. japonicum*.

In the petiole of *L. dichotomum* the structure is of the same type as in *L. japonicum* in the corresponding region, and the shape of the xylem-mass undergoes similar changes according to the level. Quite at the base of the petiole the xylem has an oval outline; at some little distance from the base (e. g. at

about one inch) it has a well-marked prominence on the morphologically lower side, while the outline of the xylem on the upper side is rounded, and the two lateral prominences are broad but only slightly raised. At a still higher level the xylem becomes three-lobed, much as in Fig. 4. In this upper region of the petiole spiral tracheides occur in connexion with all three prominences; one group near the lower corner of each lateral prominence, and one near each corner of the lower one. Towards the lower region of the petiole the two groups of spiral tracheides belonging to the lower side of the xylem are retained, but the other groups disappear, all the small peripheral tracheides of the lateral prominences being then scalariform. At a lower level still, close to the base of the petiole, spiral elements are absent altogether. The lower prominence is no longer broad and flattened, but forms a slight rounded angle with a single group of small scalariform protoxylem-elements forming its apex. This group becomes less and less marked as one passes downwards, so that the leaf-trace in passing through the cortex of the stem has a xylem of circular and then oval outline, and a nearly uniform layer of phloem surrounding the xylem. The position of the lower group of scalariform tracheides is still just distinguishable, and one is able to determine that the bundle does not undergo any great change in orientation during its passage through the cortex. The xylem of the leaf-trace passes in about radially until it is almost touching the stele, when it curves downwards and its tracheides join those of the stele. As stated above, there has been no important change in orientation, so the lower group of scalariform tracheides is probably continuous with some of the protoxylem-elements of the stele, though this cannot be stated with certainty. The node in longitudinal section shows a peculiar structure. A large number of branched tracheides occur, chiefly of a V- or Y-shape¹. They are arranged one above another and more or less fitting into one another, and are so placed that one arm of each tracheide

¹ A few similar tracheides occur at the node in *Trichomanes radicans*.

runs obliquely into the base of the leaf-trace, while the other arm is directed longitudinally forward in the stele. In this way the junction of the xylem of petiole and rhizome is effected. One of these nodal tracheides isolated by maceration is shown in Fig. 6. They occasionally have a more complicated and irregular form. Similar branched tracheides occur at the division of the stele when the stem dichotomizes.

In the petiole of *L. dichotomum* as in *L. japonicum* the proto-phloem forms a nearly continuous ring. The biggest sieve-tubes of the metaphloem and the greatest thickness of phloem occur in the three bays of the xylem. Spiral protoxylem-elements are present on the lower side of the xylem-mass. Examination of a young stage of the petiole proved that in the region, where the upper side of the xylem is rounded (without a median bay), two protoxylem-groups consisting of spiral elements on the lower side of the xylem were the first to differentiate. A good deal later two to three groups of 'protoxylem,' consisting chiefly at any rate of scalariform tracheides, were differentiated at each side of the xylem-mass, in the regions corresponding to the lateral prominences. Then subperipheral and peripheral tracheides were formed on the upper and lower sides, the elements opposite the two bays being late in differentiation. The development of tracheides in the interior is rather irregular, but appears to depend partly on the size of the mature elements to be formed. Siliceous nodules with a granular texture are fairly numerous in the xylem-parenchyma.

L. pinnatifidum has a petiole of the same type as the last species, but the bundle is smaller. The development is evidently fairly similar. In the region where the upper side of the xylem is rounded there are four protoxylem-groups, two on the lower side and two lateral. Their differentiation is probably more nearly simultaneous than in *L. dichotomum*.

In *L. scandens* the xylem of the petiole has a more rounded form than in the species described above. There are three protoxylems, one on the lower side and two lateral. Some

very thick-walled sieve-tubes are present, and resemble fibres, but were not lignified in the cases examined.

The petiole of *L. palmatum* is small compared with that of *L. dichotomum*, &c., and is of a somewhat different type. The xylem is shaped rather like an equilateral triangle with one protoxylem at each corner. The protoxylem-group on the lower side is distinctly not peripheral, but slightly embedded in the xylem. This fact and the peculiar rosette-like arrangement of the adjacent tracheides round the protoxylem-groups in this species were noticed by Prantl ('81, p. 27). Especially in this species the rule given by Bertrand ('81, p. 15), that in sections of appendicular organs the bundles show symmetrical arrangement in relation to a single straight line, does not at first sight appear to hold good, as the bundle has a nearly complete radial symmetry. But it must be noted that the median protoxylem ('centre de développement') is not quite peripheral, and that in *L. dichotomum*, at any rate, the bundle attains a bilateral symmetry at the base of the petiole by the disappearance of the lateral protoxylems.

There are three fair-sized bays of phloem, and the proto-phloem is continuous. The pericycle is from one to two cells thick, being one cell thick opposite the protoxylem-groups, and separated from them by parenchyma.

The petiole or rachis of *L. lanceolatum*, Desv., is of the usual type, except that it contains a large number of fibres chiefly in the neighbourhood of the protoxylem-groups. They are strongly lignified, and almost certainly produced by sclerosis of sieve-tubes. In addition, some of the large meta-phloem sieve-tubes situated in the bays of the xylem become lignified in different degrees. The chains of parenchyma-cells in the xylem have the walls separating them from one another considerably thickened and lignified. In the petiole of *L. heterodoxum*, Kze., the xylem-parenchyma also tends to be thick-walled, and fibrous sieve-tubes are present, but are not lignified. In *L. volubile*, while the petiole is typical, the rachis shows a structure approaching that of *L. palmatum*.

LYGODIUM, root.

The root of *L. dichotomum* has a diarch xylem-plate with sometimes two specially large tracheides in the metaxylem, as occurs in many other Ferns. The tracheides at each end of the xylem plate, including the protoxylem and a few other tracheides, are somewhat spread out as a tangential row of elements at right angles to the plane of the diarch plate. The two protoxylem-groups consist of a few small tracheides, and are in the usual position. After their differentiation the subsequent order of development of the xylem is tangentially outwards for the one or two larger tracheides completing the tangential rows mentioned above, and at the same time inwards (towards the centre of the root) for the remainder of the metaxylem. The development here and comparison with the structure in other species do not appear to give any grounds for distinguishing by a separate term the tangentially developed tracheides from the more central part of the xylem. Hence 'metaxylem' may be used for all the xylem other than protoxylem, as in the previous paper (Boodle, '00, p. 458). The endodermis consists in most cases of six flattish cells, whose radial walls correspond roughly with those of the cortical layer next outside; this layer also consists of six cells, which measure tangentially two to three times their radial depth, and have brown walls. Outside these cells there is a sudden change to many-celled layers, of which the next 1-3 layers are thick walled (according to the age of the root). The innermost of these may consist of very thick-walled cells with narrow radially elongated cavities. The remaining five or six layers of the cortex have comparatively thin brown walls. The pericycle is one cell thick in most places, and is a many-celled layer, but appears to have been formed by subdivision of a six-celled layer, whose radial walls at first corresponded with those of the endodermal cells.

The root of *L. pinnatifidum* is fairly like that of the species just described, but the endodermis consists of twelve or more cells. When the root-stele passes off from the stele of the

rhizome, it has a nearly longitudinal course until after passing through the phloem region of the rhizome. It then curves out more sharply. In *Lygodium japonicum*, at the junction of the xylem of the root with that of the stem, a few branched tracheides occur, but they differ from those at the node, in that their arms are scarcely divergent. This depends on the angle at which the root-stele separates. In all the species 'root-tracheides' are differentiated at the periphery of the stele a short distance before they 'pass out' to the root.

LYGODIUM, seedling.

The seedling¹ of *Lygodium japonicum* may now be described. The first leaf is simple and palmatipartite; the second leaf has its lamina dichotomously divided into two palmatipartite segments. In the next few leaves the petiole forks into two at the top with one pinnately lobed pinna on each fork. The later leaves have the growth of the rachis continued above the two pinnae as a circinate tendril-like projection or a bud. The appearance of the rhizome in oldish seedlings suggests that, after the first dichotomy of the rhizome, one branch does not develop far, and that growth is continued by forking of the other branch. The roots show distichous arrangement.

The structure of the seedling was examined by means of microtome-series. Several series were made, and showed differences in detail, but agreement in the main characters. The primary root is diarch. In the region of the foot the root-stele curves sharply (nearly horizontally) inwards towards the prothallus. Fig. 8 shows the arrangement of the foot (*f.*) and prothallus (*p.*) at the point where the root-stele, whose diarch xylem is shown diagrammatically at *x.*, is just beginning to curve inwards. After curving in, it turns upwards so as to become vertical again, and generally by that time has lost the distinctive characters of root structure,

¹ 'Sporeling' is of course the more reasonable term, but 'seedling' appears customary.

and may be regarded as belonging to the stem. To take one case as an example for the details:—during the double bending of the stele, the xylem-tracheides become more nearly uniform in size, and increase in number from 15 to about 36, at the same time the outline of the xylem becomes roughly circular, definite protoxylem-groups are not distinguishable, and the phloem becomes practically continuous. Then, to return to the general description, a few parenchyma-cells appear in the xylem, and a little higher up they increase in number, so that the structure of the rhizome of a mature plant is represented on a small scale, as seen in Fig. 9. This is from a section below the first leaf; *p.* is one of the xylem-parenchyma-cells. In Fig. 10 the xylem of the first leaf-trace has just separated from the xylem of the stele. From two to three leaf-traces and from one to three roots are given off (in the order of first a leaf-trace and then a root), the stele meanwhile retaining the type of structure shown in Fig. 9. After this the stem dichotomizes and leaves and roots are produced by the two branches of the stem, which grow as rhizomes. One of them is usually horizontal, while the other is inclined upwards, and, as stated above, one of them tends to be arrested. At this first dichotomy the branch cut transversely near its base shows the same type of structure as the rhizome of the mature plant; parenchyma is scattered among the xylem, and the largest tracheides are towards the centre of the latter, while the protoxylem is peripheral or nearly so. To return to one of the series for the orientation: the first leaf-trace separates from the stele on the side away from the prothallus, about in the position of an original root-protoxylem. The second leaf passes off towards the prothallus at a divergence of about 120 degrees. The stele of the stem elongates in a plane about perpendicular to one which includes the two leaves, and then dichotomizes. Each branch gives off a root on its lower side, and one branch close to its base produces a leaf-trace on its upper side.

It is clear from the above description that the structure

typical of the rhizome of *Lygodium* is attained in the seedling without any intermediate more complicated structure. Hence there are no grounds for assuming a reduction to have taken place.

With regard to the first and second leaf-trace of the seedling, although they pass off from the stele as collateral bundles, their phloem, though only containing a few sieve-tubes, spreads towards the inner side as they pass through the cortex of the stem, so that they become concentric or very nearly so, and the same structure appears to be continued in the petiole.

The leaves are radially arranged on the upright stem of the seedling, the dorsiventral arrangement of the mature plant beginning after the first dichotomy of the stem.

SCHIZAEA, habit.

The stem of *Schizaea digitata*, Sw., may be erect or obliquely ascending, or often in old plants the lower part of the stem is erect, and the upper part ascending or even horizontal. The leaves are crowded on the stem, and the roots attached between them; the phyllotaxy is difficult to determine and probably variable, but in some stems it appeared to be $\frac{1}{8}$ in places, but usually the orthostichies numbered more than three. In *S. fistulosa*, Labill., the leaves are, at any rate sometimes, dorsal and distichous. The leaves of *S. digitata* are narrow-linear, and grass-like, often over a foot in length. The petiole is triangular in section, and passes gradually into the lamina, which is flattish only in its upper region, where it shows a prominent midrib on the lower side. The leaves of certain other species are much broader and are dichotomously forked.

SCHIZAEA, stem.

In the rhizome of *Schizaea digitata*, Sw., the stele shows a marked difference from that of *Lygodium*. The distinguishing character is that the xylem consists of tracheides with usually no parenchyma among them, and forms a ring

surrounding a central pith. The ring of xylem is interrupted by the departure of the leaf-traces as seen in Fig. 11, which represents the node of *S. dichotoma*, Sw.

The rhizome of *S. digitata* has a cortex of 6-8 layers of brown sclerotic cells. The endodermis contains mucilage, and the pericycle is usually 2-3 cells thick and its cells are rather thick-walled. The outer pericyclic cells generally have their radial walls coinciding with those of the endodermal cells, and the sieve-tubes sometimes correspond with the inner pericyclic cells. The phloem consists of sieve-tubes and parenchyma, and is 2-4 or 5 cells in thickness. The sieve-tubes are sometimes tabular and three or four of them may form a neat radial row, at other times they are smaller, more rounded and irregularly placed, with parenchymatous cells among them. Some little doubt must be expressed as to the nature of the elements here spoken of as sieve-tubes. In longitudinal section they are at first sight considerably unlike the sieve-tubes of *Lygodium*. Some of them are short elements, of the same length as the pericyclic cells or slightly longer, with straight or slightly inclined end-walls; but others are a good deal longer and may have long pointed ends. The points in which they resemble the sieve-tubes of *Lygodium* are best seen in the more elongated elements, in longitudinal sections unstained and mounted in glycerine-jelly, and are as follows:—the walls are rather thick and refractive, and oval or rounded pits are seen on some of the longitudinal walls, and bear refractive granules. The phloem-parenchyma-cells have rather scanty contents, except for the presence of a distinct nucleus, and their walls do not stain quite so strongly with haematoxylene as those of the sieve-tubes. One meets with doubtful elements, which might be either sieve-tubes or phloem-parenchyma, but those which most suggest sieve-tubes are usually destitute of a nucleus.

The 'sieve-tubes' occupy the usual position for sieve-tubes, are differentiated early as in other Ferns, and also when first formed are well differentiated by haematoxylene. All the characters point to the elements in question being sieve-

tubes corresponding to those of other Ferns, but probably mixed with elements intermediate between sieve-tubes and phloem-parenchyma, and possibly somewhat reduced.

The xylem is separated from the phloem by the usual ring of conjunctive parenchyma, and forms a complete round or oval zone, which is interrupted by the departure of the leaf-trace. The xylem-ring in *S. digitata* is like that of the species figured (Fig. 11), but consists of a thinner layer of tracheides, which is usually 1-3 elements thick. The tracheides are scalariform with narrow pits, and are usually rather short, and often irregular. As in *Lygodium*, there are no definite protoxylem-groups, but the first formed tracheides are scattered. With such a thin ring of xylem it is difficult to determine whether the xylem is endarch or exarch, but Fig. 12, which represents the young node, shows that the development is irregular; *a.* and *b.* are examples of an outer and an inner tracheide respectively being the first to differentiate. Protoxylem-formation in this case progresses from one side of the stem to the other.

Within the xylem-ring is the pith, the cells of which are sclerotic, contain mucilage, and have numerous fine pits in their walls. The walls of the pith-cells adjoining the xylem are usually thinner than the rest, and remain unlignified longer, and in these cells mucilaginous contents are less or absent. There is no internal endodermis. In the region of the leaf-gap the pith becomes continuous with the pericycle, and its cells, especially the outer ones, resemble those of the pericycle, which become fairly thick-walled and occasionally show pitting like that in the pith. The inner cortical cells also possess pits just like those of the pith-cells, but their walls are brown. The pith-cells are not very much elongated, being shaped like rather long parenchymatous cells and having often flat ends.

There is a tendency to lignification in the pericycle and phloem. It was found by treating a section with phloroglucin and hydrochloric acid that the tracheides were very strongly lignified. The pith-cells were fairly strongly lignified,

except the outermost layer of the wall, which was not lignified; but the peripheral cells of the pith had hardly more than the middle lamella lignified. In the pericyclic cells the wall at the corners was often lignified, sometimes the middle lamella as well, or sometimes the greater part of the wall. The sieve-tubes sometimes had their walls lignified at the corners. The lignin stain was not seen in the sclerotic cortex. The middle lamellas of the cortical cells, pith and pericycle were curiously resistant to sulphuric acid, and were not dissolved by boiling for a short time in potash-solution.

The stem-structure of *S. dichotoma* and *S. fistulosa*, Labill., was examined in fragments of dried material. The figure *S. dichotoma* (Fig. 11), and the diagram of the same species (Fig. 15) will be referred to in the description of the node. The structure of *S. dichotoma* (Fig. 11) need not be described at length, as it is of the same type as *S. digitata*, but it should be mentioned that one or two fibrous sieve-tubes, apparently lignified, occurred in the phloem near the two ends of the arc of xylem belonging to the stem. The smaller tracheides are usually at or towards the periphery of the xylem. Two or three layers of thin-walled cells with small intercellular spaces at the corners lie between the brown outer part of the cortex and the endodermis.

In *S. fistulosa* there is a broad cortex consisting of fairly thin-walled cells, of which the outermost one or two layers only are coloured brown. The pith-cells are also fairly thin-walled, but have collenchyma-like thickenings at the corners. The xylem is only 1-2 tracheides thick, but where it is two-layered, if the tracheides differ in size, the smaller ones are nearly always at the outside; so the structure tends to be exarch. Another piece of rhizome of this species was probably a short distance behind a dichotomy, as it had two nearly equal-sized steles, both of which were slightly irregular, and the xylem was more or less interrupted by leaf-gaps. The two steles had been giving off leaf-traces; three had been derived from each stele and were found at different distances on their way out through the cortex.

In *Schizaea digitata* a comparison of transverse sections of the young stem at different stages of development points to a possibility that the endodermis, pericycle and phloem may be formed by subdivision of a single layer. This would be a very remarkable case of stelar endodermis, or cortical phloem, and it is intended to reinvestigate it.

SCHIZAEA, petiole and node.

A transverse section of the petiolar bundle of *Schizaea digitata* is represented in Fig. 13. The bundle is seen at once to be collateral. There is a band of xylem on the morphologically upper side, with no well-marked protoxylem, but judging by the size of the tracheides those laterally placed are probably the first to differentiate. This section was cut near the base of the petiole, and spiral elements appear to be absent in this region, the tracheides being scalariform. The phloem forms a band on the lower side (*ph'*), and consists of thick-walled sieve-tubes and a certain amount of parenchyma. A group of fibres (*f*) is seen touching the xylem at each end of the bundle. Their end-walls resemble sieve-plates and they appear to represent lignified sieve-tubes. The pericyclic cells are rather thick-walled as in the stem, but have been represented as thin-walled in the figure. Sections of a young petiole cut higher up than the level of the section just described, prove that spiral protoxylem-elements are formed on the upper side of the bundle roughly in the median region (Fig. 14, *px'*) and the differentiation proceeds outwards on both sides along the periphery of the xylem, so that a crescent of tracheides is formed, the more centrally placed tracheides developing subsequently. The nearly median first-formed tracheides become crushed rather early, and are scarcely distinguishable in the mature petiole. In the lamina of *S. digitata* the bundle in the midrib resembles the bundle of the petiole, but has more numerous fibres. In the median region on the lower side, in the sections examined, three pericyclic cells had become much enlarged, and had crushed the greater part of the

phloem. If normal, this may be compared with 'cavity-parenchyma.'

At the node a segment of the xylem-ring separates for the leaf-trace, and carries with it the phloem on its outer side. The leaf-trace is seen cut rather obliquely in Fig. 12. In *S. dichotoma* the leaf-trace is seen separated from the xylem and phloem of the stem in Fig. 11, but still enclosed by the endodermis. Fibres occur in the leaf-trace. In the diagram, Fig. 15, the leaf-trace surrounded by its own endodermis is on its way out through the cortex, and the endodermis of the stele has dipped into the leaf-gap as far as the inner limit of the xylem. As far as was observed, the pith remains separated from the cortex by the endodermis in the nodal region. The xylem of the leaf-trace becomes detached from the xylem of the stem at one end first. In *S. fistulosa* the leaves are dorsal and apparently distichous, but in the specimen described above as preparing for dichotomy the phyllotaxy was $\frac{1}{3}$.

In *S. fistulosa* some of the leaf-traces in the cortex had an arched xylem-mass, with its convexity directed outwards. This type of structure is an approach to what is found in *Anemia*. The petiolar bundle resembles that of *S. digitata*.

SCHIZAEA, root.

In the root of *Schizaea digitata* there are only three layers of cells outside the endodermis. The outermost of these is the piliferous layer, and often peels off in the old root, then follows one layer of thin-walled cortex, and one layer of cells with their inner and radial walls very strongly thickened. In old roots the stratification of these walls is remarkably distinct. The endodermal cells are flattish. Both pericycle, endodermis and the layer of sclerotic cortical cells usually consist of six cells each, but sometimes of eight or nine cells, especially in the case of the pericycle. The xylem forms a typical diarch plate, with two large tracheides in the metaxylem. The structure is nearly identical with that

figured by Prantl ('81, Fig. 59) for *S. pennula*, Sw. The radial walls of the pericycle, endodermis and sclerotic layer of the cortex correspond, while the walls of the next cortical layer, which may consist of twelve cells, do not coincide with them. The pericycle therefore appears to be cortical.

ANEMIA, habit.

In *Anemia Phyllitidis*, Sw., the stem is obliquely ascending, the leaf-bases are crowded and leave scarcely any free surface to the stem. The roots are borne by the stem in longitudinal rows between the leaf-bases, and stick out through the narrow fissures left between the latter (Prantl, '81, p. 2). The phyllotaxy appears to be $\frac{2}{5}$ as stated by Prantl. Polystichous leaf-arrangement occurs in most species of *Anemia*, but seven species have a creeping rhizome, which bears distichous leaves, sometimes distant, on its upper side. These species are included by Prantl in the subgenus *Ancimiorrhiza* (*Anemiorrhiza*, J. Sm. emend.), and the above characters form part of the diagnosis of the subgenus. *A. mexicana*, Klotzsch., and *A. cuneata*, Kze., are examples of this type.

The leaf in the genus is petiolate, and pinnate, the pinnae being nearly entire, pinnately lobed, or again pinnate in their basal region.

ANEMIA, stem.

Fig. 16 is a diagram of a transverse section of the stem of *Anemia Phyllitidis*, the vascular tissues or rather their endodermal sheaths being shown in outline. The structure is entirely different from what has been described in the stems of *Lygodium* and *Schizaea*, and may be called dialystelic, and the masses of vascular tissue represent steles, or meristeles, or bundles, according to what view one takes of their homology and of suitable terminology. In the particular case figured two leaf-traces (viz. *l.z.* and the bundle on the opposite side of the stem) are seen on their way in towards the interrupted vascular ring, which here consists of two parts. These latter represent the section of a hollow reticulate vascular

cylinder similar, except as to leaf-traces, to that of *Nephrodium Filix-mas*, Rich. In *Anemia* each mesh is a leaf-gap, and the leaf-trace is inserted about at its base as shown in Prantl's diagram (Fig. 27 A).

In the transverse section of the stem the ground-tissue appears as a uniform tissue of rounded, starch-containing, parenchymatous cells frequently with small intercellular spaces at the corners. In longitudinal section these cells are from two to six times as long as broad, and have flat or sometimes pointed ends. Three or four layers at the periphery may become sclerotic. In Fig. 16 two roots (*r.*) are seen on their way out through the ground-tissue. A cavity or pocket (not shown in Fig. 16) is present in the ground-tissue on the inner side of one of the incoming leaf-traces. A pocket which communicates with the exterior is present in connexion with each leaf. It is axillary in position, slit-shaped where the petiole joins the stem, but becoming triangular and decreasing in size in passing downwards, and disappearing before the leaf-trace joins the vascular cylinder. Three of these axillary pockets are represented in Prantl's Fig. 27 B. The tissue limiting them bears numerous hairs, and becomes sclerotic. The structure of the leaf-traces and of a large part of the vascular cylinder may be best described in reference to the petiolar bundle, but the structure of one of the flattish 'steles' forming part of the vascular ring in some sections may be described here, as more characteristic of the stem. A section of one such stele is really one of the strands composing the reticulate cylinder, cut near the base of a mesh. It has an elongated elliptical outline formed by the rather flat-celled endodermis (*e.* in Fig. 40, which represents a small piece of the stele). The pericycle forms a continuous layer about two cells thick, while the phloem consists of a band of sieve-tubes on the outer and inner side of the xylem (*ph.* and *i. ph.* in Fig. 40). On the inner side of the xylem the sieve-tubes form a continuous or nearly continuous band from one to two elements thick, but on the outer side of the xylem the sieve-tubes are less numerous and smaller and form only a broken

band, interrupted by parenchyma. The sieve-tubes are not present at the ends of the xylem-mass. The sieve-tubes are in most places separated from the xylem by conjunctive parenchyma, 1-2 cells thick; this tissue also is better developed on the inner than on the outer side.

The xylem occupies the centre and has nearly the same form as the whole strand. In this region it consists entirely of scalariform tracheides and parenchyma; spiral elements being absent. As seen in Fig. 40 the smaller tracheides are at or towards the outside (on the right in the figure), the largest ones being at the inner side. At the two ends of the xylem-strand there are rather numerous small tracheides taking up the greater part of the thickness of the xylem. Some of the small tracheides scattered along the outside of the xylem-band together with some at the ends, and one or two immersed in the xylem, are the first to differentiate. So the strand has rather irregular development, but a tendency to the exarch type¹. Root-steles are attached to the ends of the stele, where the numerous small tracheides are present; a root may fuse with one end of the strand, just before the attachment of a leaf-trace to that end.

The scalariform tracheides are of a common type, with the pits often running the whole width of the wall. The sieve-tubes have inclined end-walls, and the sieve-plates are crowded on the longitudinal walls of the larger elements so as to give them a reticulate appearance. The strips of wall between the sieve-plates are narrow, but sometimes thickened to a remarkable extent, and the thickenings on the two sides of a longitudinal wall may together have a dumb-bell-shape in section, so that the sieve-plates are in these cases actually bordered. Granules are seen on some of the sieve-plates. In Fig. 40 some of the small tracheides are detached from the rest of the xylem, so as to be in contact with the sieve-tubes. This is rather exceptional.

In the majority of species of *Anemia* (viz. excepting Prantl's subgenus *Aneimiorrhiza*) the structure of the stem appears

¹ That is, exarch with regard to the centre of the stem.

from Prantl's statements (p. 22) to be of the same type as in *A. Phyllitidis*. The stem of *A. Dregeana*, Kze., was examined, and found to be dialystelic, and differed only in unimportant details from *A. Phyllitidis*.

ANEMIA, petiole and 'node.'

The petiolar bundle of *Anemia Phyllitidis* is well figured and described by Prantl (Fig. 30), but an illustration is given here for the sake of completeness (Fig. 17). The xylem consists of a band of tracheides shaped like a flat-topped arch with divergent supports, which have hooked ends turned inwards. The tracheides are mostly scalariform, but three protoxylem-groups of annular and spiral elements are found on the inner surface of the xylem:—one at the median point, and one at a short distance from each hook. The hooks are formed of small scalariform tracheides. The phloem, which is usually separated from the xylem by a layer of conjunctive parenchyma, forms a layer covering the outer surface of the xylem-arch, and is continued on the inner surface about half way up each arm. It consists of sieve-tubes and parenchymatous cells scattered among them, and distinguished from them by their dense contents and larger size. The phloem is thickest on the outer and inner sides of the arms. Several of the sieve-tubes become converted into thick-walled lignified fibres, one of which is shown in Fig. 19. This element was already lignified, but its walls had not attained their full thickness, and the structure of the oblique end-wall, and also the presence of numerous refractive granules on it, are suggestive of what is found in a functional sieve-tube. These fibrous elements form a thin continuous layer along the flat top of the xylem-arch and on the convex surface of both the xylem-hooks. In these regions the phloem is thin, and practically all the sieve-tubes are replaced by fibres. Fibres also form a fairly compact mass filling the concavity of the hooks, and some occur scattered along the inner and outer face of the xylem for some little distance upward from the hooks. In all these cases the fibres are usually separated from the xylem by

a layer of conjunctive parenchyma, but several fibres may also be in contact with the xylem. The pericycle forms a continuous layer of rather large cells, and is 2-4 cells thick, but reduced to a single row of cells on the flat top of the arch and at the ends of the arms. In these regions its cells have dense contents. The endodermis is very distinct, and roughly follows the outline of the xylem. Cavity-parenchyma¹, which belongs to the conjunctive parenchyma adjoining the xylem, occurs in connexion with the three protoxylem-groups, but is most marked in the case of the median group, where two enlarged cells of this tissue may be recognized in Fig. 17. A cell belonging to the cavity-parenchyma is shown in longitudinal section in Fig. 18. It contains a nucleus and protoplasm, and has formed two large protrusions, which have grown into the space originally occupied by an annular tracheide of the protoxylem. Some of the rings of this element are seen crushed together at *r*.

The epidermis and the succeeding five or six layers of the cortex are sclerotic, and there is a gradation from the small-celled epidermis to the large-celled inner layer of this zone. The rest of the cortex or ground-tissue is thin-walled. In cells of this tissue not far outside the endodermis a few silica-nodules were met with. They were usually solid-looking, and roundish or irregular in form. Fig. 20 is one of them, and is double, with a cavity in each half.

In the basal region of the petiole the fibres become reduced in number until none are left. The top of the xylem-arch becomes more rounded, and the hooks become less incurved, until nearly absent. There are still three protoxylem-groups in the same positions as before, but, close to the base of the petiole, the median protoxylem appears to be the only one which possesses spiral tracheides, and is formed a little earlier than the other two. The ground tissue becomes fairly thick-walled, and the part of it filling up the concavity of the arched bundle becomes specially thick-walled.

The leaf-trace in the cortex of the stem resembles the

¹ This tissue will be referred to again below.

bundle in the base of the petiole in structure ; no fibres are present ; the pericycle forms a layer of nearly uniform thickness ; the phloem is continuous round the ends of the bundle, and three protoxylem-groups are present. As it passes inwards through the cortex the phloem on the inner side of the xylem spreads from both ends towards the median point, where it ultimately meets. When the leaf-trace reaches the vascular cylinder a rounded stele fuses with one end of it, producing a knob at that end ; soon afterwards a long flat stele approaches the other end and fuses with it, thus producing a long stele curved towards one end. The lower stele in Fig. 16 is an example of this. The knob is seen at the right-hand end ; the curved part is the leaf-trace, and the straight part on the left is the long flat stele just referred to. The little projection on the lower side is connected with a root, which is attached to the stele just as it fuses with the leaf-trace. Part of this flat stele remains as a knob on the left-hand end of the leaf-trace, and the remainder of the stele branches away again as a rounded stele ready to fuse with the next leaf-trace. The leaf-trace that we have been following has by this time a slightly curved form with a knob at each end, the phloem is interrupted at its ends, and the median protoxylem-group, which was present and contained spiral elements while the fusions were going on, now or a little lower down becomes lost. In passing downwards this strand becomes straightened out, and the enlargements at its ends less pronounced, while its protoxylem becomes scattered and largely external, as mentioned above when describing the stem ; that is, it gradually loses its leaf-trace characters. If one reverses the order of this series, one may say that, in following a stele upwards in the stem, it divides into three parts, of which the middle one, changing slightly in structure, becomes the leaf-trace.

The petiolar bundle of *A. Dregeana* is not very different in type from that of *A. Phyllitidis*, except that, as Prantl (p. 25) observed, fibres are absent. The xylem forms a considerably flattened arch, with slightly hooked ends. The phloem has

about the same distribution as in *A. Phyllitidis*; it is continuous over the top of the arch, and continuous or slightly interrupted at the ends of the two arms. There is a median protoxylem-group with cavity-parenchyma, and two other protoxylem-groups on the inner side of the xylem-arms; they start just above the hooks, and are spread out about one-third of the way towards the median group. On account of the flatness of the xylem-arch the endodermis only dips in slightly on the upper side of the bundle.

ANEMIA, root.

In the root of *Anemia Phyllitidis* the cortex is twelve or more cells in thickness. The outer three or four layers are comparatively thin-walled and graduate into the sclerotic inner cortex. The endodermis and pericycle are both many-celled, and the pericycle is usually one cell thick. The stele has a typical diarch structure, often with two very large tracheides in the metaxylem. The protoxylem-elements and adjacent tracheides are not spread out tangentially to the same extent as in *Lygodium*. The base of the root stele in the cortex of the stem is diarch, but the middle region of the xylem-plate is occupied by numerous irregularly placed tracheides, and the structure may be slightly asymmetrical.

ANEMIA MEXICANA, ETC.

The following species are included by Prantl in the subgenus *Aneimiorrhiza*:—*A. aurita*, Sw., *A. coriacea*, Gris., *A. mexicana*, Klotzsch., *A. cicutaria*, Kze., *A. Wrightii*, Baker, *A. cuneata*, Kze., *A. adiantifolia*, Sw. These are characterized among other things by their creeping rhizome and distichous dorsal leaves. This appears to be correct at any rate for some of these species, but certain specimens may perhaps have polystichous leaves.

A transverse section of an internode of *A. mexicana* shows a structure which differs from that of *Anemia Phyllitidis* in having a closed ring of vascular tissue instead of an interrupted

one. The structure resembles the *solenostelic* type¹ as defined by Gwynne-Vaughan ('01, p. 73), while *Anemia Phyllitidis* is dialystelic. The cortex of *A. mexicana* consists of brown elements, which are slightly sclerotic in the outer half, and strongly sclerotic in the inner half. Fig. 38 represents the stelar ring of this species, which is bounded by an outer and inner endodermis. Owing to the rupture of the radial walls of inner and outer endodermis, the cortex and central ground-tissue which is sclerotic have broken away, and only the stelar ring is shown in the figure. It is about 1 mm. in diameter, and its structure is as follows:—the pericycle forms a layer 2-4 cells thick on the inner and outer side; there is an inner and outer layer of phloem, each consisting of a practically continuous layer of sieve-tubes, mostly one element thick. The sieve-tubes are rather thick-walled, and stain deeply with haematoxylene. A layer of conjunctive parenchyma separates the phloem on both sides from the xylem. The xylem-ring is about five tracheides in thickness. The tracheides are scalariform, and the larger ones are on the inner side, the smallest tracheides being at or near the outer side. This is seen in the small piece of the stelar ring shown in Fig. 39. A comparison of this figure with Fig. 40 shows that there is a close agreement in structure between the solenostelic ring of *Anemia mexicana* and the separate 'steles' of the stem of *A. Phyllitidis*. In *A. mexicana* a spiral protoxylem-group which comes in with the leaf-trace is continued a short way down in the stem. The tracheides elsewhere in the stelar tube appear to be all scalariform. In this and other species of the same subgenus which were examined, only small pieces of dried material were obtainable, so the structure was not as fully investigated as was desirable. The petiole in this and the next species was not examined.

A. adiantifolia has in its rhizome practically the same structure as *A. mexicana*.

In *A. coriacea*, Griseb.², the solenostele is less than $\frac{1}{2}$ mm.

¹ Leaving the behaviour of the endodermis at the node out of the question.

² In Hooker and Baker's *Synopsis Filicum* (p. 433) the authors were inclined

in diameter; it is thus much smaller than in *A. mexicana*, but has similar structural details. Fig. 41 is a diagram of the structure found just below the node. The inner and outer phloem are each represented by a ring of dots; *e.* and *i. e.* are the outer and inner endodermis, separated in each case from the phloem by a wide layer of pericycle. The xylem-ring is bulged out on the right, preparatory to the separation of a leaf-trace, and *e'* is a group of three or four endodermal cells differentiated in the pericycle in this region. The shaded mass within the inner endodermis (*i. e.*) is a group of sclerotic tissue. The structure found a little higher up in the nodal region is seen in Fig. 42. The xylem of the leaf-trace has become separate, but the phloem is still attached at one end. The little group of endodermal cells (*e'*) was not well preserved, but appears to form a ring as shown in the diagram. Higher up, this ring becomes broader, until it meets and fuses with the outer endodermis, which bends inwards on either side between the leaf-trace and stele. The endodermis then splits so that the stele and the leaf-trace are each surrounded by an endodermis. In this way the continuity of the endodermal sheath is not broken. Fig. 43 shows the leaf-trace (*l. t.*) on its way out through the cortex. It has a slightly arched xylem with a protoxylem-group at the median point on the inner side. The phloem is nearly continuous, or perhaps interrupted for a short distance near the middle of the inner side. For the petiole see p. 421.

The stele of *A. cuneata* has the same type of structure as *A. mexicana* and *A. coriacea*, and is intermediate between them in size.

In *A. aurita* an unexpected structure was found in the rhizome. The stele is about $\frac{1}{2}$ mm. in diameter, which is slightly larger than in *A. coriacea*. The xylem forms a nearly solid mass of circular outline, and consists of tracheides, with a few rows of parenchyma-cells among them. The large tracheides are towards the centre, and the smaller ones at

to refer *A. coriacea*, Griseb., to *A. hirsuta*; but Mr. J. G. Baker has since seen specimens of *A. coriacea* and is of opinion that it is distinct from *A. hirsuta*.

the periphery. This structure might be taken for that of a small species of *Lygodium*, if it were not for the presence of a quite small excentrically placed group of parenchyma or other soft-walled tissue. Unfortunately this was partly disorganized and its nature could not be determined. In the petiole of this species the bundle is like that of *Anemia Phyllitidis* on a small scale, but differs as to endodermis and one or two other points. The xylem forms a V-shaped arch, the ends of which are slightly incurved, hardly hooked. There is a median protoxylem placed just as in *A. Phyllitidis*, and two other groups in much the same position as in that species. The phloem is slightly interrupted at the ends of the xylem-arch, but forms a layer covering the rest of the outside of it, and on the inner side of it reaches about a third of the way up towards the median protoxylem. Several fibres, apparently formed from sieve-tubes, occur in the phloem; a few above the top of the arch and more numerous ones near the ends of the xylem. There is a thick pericycle, and the endodermis does not dip into the concavity of the arch, but has a rounded outline.

The structure of *Anemia aurita* was not examined by Prantl, but he investigated some of the other species included by him in *Aneimiorrhiza*, and described the tubular stele as occurring in them. Robert Brown ('38-'52, p. 2) mentions having observed a complete circle of scalariform tracheides in the stem of some species of *Anemia*.

The occurrence of a solenostele-like stelar tube in one group of species, and of the dialystelic structure in the remainder, is, as Prantl states, connected with the fact that the leaves are distichous and not crowded in the first case, but polystichous and crowded in the second case.

ANEMIA, seedling.

Several series of microtome sections were made of seedlings of *Anemia Phyllitidis*, but on account of the difficulty of penetrating the sclerotic tissue with paraffin and of cutting it, most of these were incomplete. However, by comparing one

series with another, the structure of the transitional region was ascertained. By soaking one of the seedlings for nearly twenty-four hours in Eau de Javelle before embedding, a series was obtained, which was satisfactory through the greater part of its length. Figs. 21-31 (excluding Fig. 23) are sections of the axis in ascending order. Fig. 21 shows the structure of the primary root. It is of the typical diarch type, but is not quite mature. One point, however, in which the majority of Ferns would perhaps show a difference, is that here the first-formed phloem-elements (*pph.*) are adjacent to the protoxylems, so that there are four protophloems. The same mode of development is also found in the lateral roots of the young plant. The root stele bends in towards the prothallus in the region of the foot, and becomes vertical again, just as in *Lygodium*. It may then or soon afterwards be called the stem-stele, as it has lost the characteristic root-structure, and its xylem consists of a solid mass of tracheides of fairly uniform size. The first leaf-trace is then given off (Fig. 22), and the xylem of this leaf-trace (*x'*.) consists of only about six tracheides. One or two small ones which were crushed are not shown in the drawing. Fig. 23 is the xylem of the same leaf-trace on its way out through the cortex. Soon after the separation of the first leaf-trace, a parenchyma-cell appears inside the xylem (Fig. 24). It may be connected by one or more obliquely placed cells with the conjunctive parenchyma surrounding the xylem at a lower level, and not with the leaf-trace; e.g. the parenchyma-cell seen in Fig. 24 had a downward connexion of this kind on the side away from the leaf-trace. The second leaf-trace is given off leaving the stele much as in Fig. 24. Then two or three more soft-walled elements appear adjoining the first parenchyma-cell in the xylem, and of these one or two are probably sieve-tubes. These immersed elements change their position slightly, so that the xylem becomes open, and one of them comes into contact with the external conjunctive parenchyma. A stage of this kind is shown in Fig. 25, where the two elements embedded deepest in the xylem are probably sieve-tubes,

and the cells marked with crosses are endodermal cells. The first lateral root-trace joins the stele, and the central parenchyma and sieve-tubes keep changing their position slightly so as to be again connected with the outer parenchyma and again shut in. The third leaf-trace passes out, the parenchyma remains enclosed, and the second root-trace joins the stele. The central soft-walled tissue increases and the xylem opens. Fig. 26 illustrates this stage. The four elements marked with crosses are almost certainly sieve-tubes, the other elements immersed in the xylem being parenchyma. Thus the central tissue at this stage is to be regarded as phloem. The fourth leaf-trace is then given off, leaving the xylem open, and the third root-trace is attached. The parenchyma then tends to form a more rounded mass with the sieve-tubes in the middle of it, and they here have a connexion with the external sieve-tubes. After the exit of the sixth or seventh leaf-trace the xylem closes and a parenchyma-cell appears in the middle of the central group of sieve-tubes. Then several other parenchyma-cells are formed adjacent to it, so that the stage shown in Fig. 27 is reached. This is where the eighth or ninth leaf-trace is preparing to pass out, and in this figure *x'* and *ph'* are the xylem and inner phloem which will pass out in the leaf-trace. After this one or two endodermal cells appear in the central parenchyma, and a little higher up are connected (the xylem being now open) by a single row of endodermal cells with the outer endodermis. This is shown diagrammatically in Fig. 28. The connexion is almost at once broken again; one or two endodermal cells remain in the central parenchyma, increase in number, and a parenchyma cell is formed in the middle of the endodermal group. Fig. 29 is a diagram of this stage, and Fig. 30 is a drawing of part of the section showing the ring of endodermis and the adjacent tissues. This endodermal ring or group, at a later leaf-gap, becomes connected with the outer endodermis by a double row of endodermal cells so that the horseshoe-shaped stele of Fig. 31 is formed. The incurved part of the endodermis (*e.*) surrounds a projection of parenchymatous tissue resembling

the ground tissue outside. When the next leaf-trace passes off the horseshoe-shaped stele becomes broken in its arched region so that two sausage-shaped or reniform 'steles' are formed, each surrounded by an endodermis. These may be repaired again to form a horseshoe-stele once more, but at the next leaf-trace the two separate steles are re-formed, and subsequent leaf-gaps cause further division of them, and they become more distant from one another so as to form a broken ring round a fairly large mass of central ground-tissue. The mature stem-structure is then attained. Differences occur in different seedlings as to the size of the xylem, the number of central phloem elements, and the stage reached at the level of a certain leaf-trace, but the above description may be taken as recording the successive changes in the arrangement of the tissues. The main facts as to the continuity of the tissues up to the horseshoe-stage of the stele agree with what is found in another dialystelic Fern, *Pteris aquilina*, as described by Leclerc du Sablon ('90, p. 3) and Jeffrey ('00, p. 9).

The transitional region of the seedling having been described, a few remarks may be made on other structural points in the seedling. It could not be clearly made out whether the first one or two leaves had a collateral or a concentric petiolar bundle, but the bundle in the petiole of an early leaf (Fig. 32) shows the phloem (*ph.*) surrounding the xylem. There is probably only one protoxylem group, the median one (*px.*), and the xylem forms a very slightly arched band. A few fibres (*f.*) are found in the phloem. Fig. 33 shows the petiolar bundle of a later leaf in an early stage of development. The median protoxylem-group (*px.*) and two protophloems (*pph.*) are differentiated. The incompletely differentiated phloem elsewhere is small-celled, and it can be seen that it is interrupted in the median region of the morphologically upper side, as in the leaves of the mature plant. Fig. 34 is a young stage of one of the steles in the dialystelic region of the stem in the seedling. The first formed tracheides are scattered. The first three or four leaves of the seedling are simple, with

a nearly orbicular, or ovate cordate lamina. The next leaf is tripartite.

MOHRIA, habit.

The stem of *Mohria caffrorum*, Desv., is an ascending or nearly horizontal rhizome, and bears polystichous leaves which appear to have a $2/5$ phyllotaxy, as in *Anemia Phyllitidis*. The leaves are bipinnate, that is, in the lower region of the pinnae. The genus includes about three species. Christ ('97, p. 352) describes it as 'vielleicht der auffallendste aller Farne, indem er die Fructification einer so weit entlegenen Gruppe mit der Organisation einer Pteridee vereinigt.' In anatomy, however, *Mohria* shows close relation to the type of structure of species of *Anemia*.

MOHRIA, stem.

Prantl ('81, p. 26) states that *Mohria* agrees completely in the structure of its strands with the majority of *Anemias*, only the fibres are absent. This is the case, as the points of structural differences between it and the dialystelic *Anemias* lie chiefly in unimportant details such as the distribution of sclerenchyma in the stem and petiole, and the outline of the bundles.

In the mature stem of *Mohria caffrorum* the epidermis is thin-walled and contains mucilage. The outer cortex or ground-tissue is fairly thin-walled, and is followed by a zone of brown sclerotic tissue which surrounds the vascular cylinder, but has an irregular form, as it curves outside the incoming leaf-traces. The central tissue within the vascular cylinder also consists of brown sclerotic elements. Fig. 35 shows the arrangement of the vascular tissue in a transverse section. This is drawn from a young stem in which the tissues were not mature, so the sclerotic ground tissue is not yet differentiated. In this figure, *sc.* is a group of sclerotic tissue filling the concavity of a leaf-trace. In the mature stem one such group, which is continuous with a similar one in the petiole, accompanies each leaf-trace through the cortex, and

finally abuts on the mass of sclerotic tissue occupying the centre of the stem. No axillary pockets are present. The leaf-trace on entering the stem has the form of a low curved arch, then becomes U-shaped as it passes inwards, and fuses at its ends with two adjacent steles in much the same way as in *Anemia Phyllitidis*. *a, a* are two steles, which will fuse with the leaf-trace opposite them; the larger one corresponds with the long-flat stele described in *Anemia*. After fusion the bundle has a conspicuous knob at each end (Fig. 35, *b.*), as in *Anemia*, and soon becomes less curved, and finally the whole has an oval outline. The changes with regard to the phloem and protoxylems are nearly the same as in *Anemia Phyllitidis*, but the leaf-trace in passing through the cortex often has five protoxylem-groups, one median one, which includes spiral elements, and is a little earlier than the others, two close to the ends of the xylem-arch, and one about halfway up each arm of the xylem. This, however, sometimes occurs in *Anemia*. The steles are smaller than in *Anemia Phyllitidis*, but have a similar structure. The tracheides are scalariform, and the sieve-tubes sometimes have very prominent thickenings between the sieve-plates. Fig. 37 is a longitudinal section of a stele in the young rhizome, at a level where hardly more than protoxylem was differentiated. It shows two sieve-tubes (*ph.*) separated from one of the early tracheides by conjunctive parenchyma.

MOHRIA, petiole.

The petiolar bundle of *Mohria caffrorum* with the adjacent group of sclerotic tissue (*sc.*) is shown diagrammatically in Fig. 36. The distribution of the phloem is shown by the shading. The xylem forms an arch with hooked ends, and there are three protoxylem-groups: one median, and one just above each hook. They are on the inner surface of the arch, and each is accompanied by cavity-parenchyma. Excluding the sclerotic tissue, which does not belong to the bundle, the structure is almost indistinguishable from that of

Anemia Dregeana, except for the fact that in the latter species the lateral protoxylem-groups are spread out, while in *Mohria* they are small. Fibres are absent in both.

MOHRIA, root.

The root of *Mohria caffrorum* agrees with that of *Anemia Phyllitidis* in the structure of its cortex, endodermis and pericycle, the chief point of difference being in the stele, which in the roots examined did not have two specially large tracheides in the middle of the metaxylem, but the xylem-band showed more gradation from the small elements at its ends to the larger tracheides in the middle, which were fairly numerous and irregularly placed. This distinction is however not very important, as the *Mohria* type of structure is sometimes found in species of certain Polypodiaceae, which usually have two large tracheides in the metaxylem, as in *Anemia*. The root of *Mohria* resembles somewhat the basal region of the root in *Anemia*.

CAVITY-PARENCHYMA.

This tissue, which is well developed in *Anemia Phyllitidis*, has been described in that plant by Prantl ('81, p. 24). A similar tissue was found by Gwynne-Vaughan ('01, p. 87) in *Loxsonia*, and his term (cavity-parenchyma), which is an equivalent for Russow's Lückenparenchym, has been adopted here. Reference to other authors who have described a similar tissue are given by Prantl and Gwynne-Vaughan. It is found in a large number of Ferns belonging to different Natural Orders, e. g. besides the two genera just mentioned, in *Helminthostachys*, *Matonia*, *Osmunda*, *Angiopteris*, *Cyathea*, *Pteris*. The character of this tissue, as described by different authors, lies in the fact that the cells composing it become enlarged and produce thylosis-like swellings or branches. As mentioned by Gwynne-Vaughan, they grow into the space caused by the disintegration of the protoxylem-elements. They may perhaps have a mechanical function. In *Tricho-*

manes Prieurii the cavity-parenchyma becomes lignified, and one element of this tissue was noticed, which was much elongated, fibrous, and pitted like other fibres in the bundle, but with one or two protrusions on the side on which it was in contact with an annular tracheide.

SIEVE-TUBES.

In examining the sieve-tubes of the Schizaeaceae aniline blue and corallin-soda were at first used for the purpose of determining whether callus was present. These stains were however discarded¹ in favour of azo-blue, which was found to be preferable for several reasons. It may be stated at once that no callus was found in any of the Schizaeaceae examined, but, as it was found by experimenting with other plants, that azo-blue is an excellent stain for differentiating callus, it will be as well to describe the method of using it. Poirault ('93, p. 139) recommends the use of azo-violet as a specially good stain for callus. Its action appears to be similar to that of azo-blue, for Mangin ('90, p. 120) classes the two together with other dyes, and states that they are generally precipitated by acids, but stain cellulose and callus in a neutral or slightly alkaline solution.

To prepare the stain, a little of the solid (Grübler's azo-blue) is stirred up in water. It is practically insoluble, but in suspension has somewhat the appearance of a violet solution. A little caustic-soda or potash-solution is added until the colour becomes reddish, the solution is then shaken up and filtered, and a fairly dark red solution is obtained, which is the reagent for staining callus. This solution when exposed to the air gradually loses its alkalinity owing to carbonation of the potash, and finally turns violet, the stain being then precipitated. One can judge of the relative alkalinity of the red solution by the length of time a little of it in a watch-glass requires before assuming a violet tinge at the periphery. The dye gives the best results in a fairly strong but only

¹ Staining deep enough to colour very small masses of callus was found to incur a danger of staining proteid granules as well.

slightly alkaline solution. When testing for callus it is advisable always to work with a section of *Cucurbita* used as a control, to be stained in the same watch-glass with the other sections. This shows at once whether the dye is working properly. If the staining solution is strongly alkaline, it will stain all parts of the section, but if rightly used it produces remarkable differentiation, when any callus is present. Sections may be left for ten minutes or more in the stain, according to its strength. They are then transferred to a slide, the stain is drained off, and a few drops of water are added until the sections change from red to lilac or bluish or nearly colourless, as the case may be. They are then examined at once, and the callus if present is found to be crimson, while cellulose walls are generally lilac or blue, but proteid and also lignified walls are entirely uncoloured. In time the callus turns violet and its colour finally fades, but when first mounted as above, the callus is the only red-stained substance in the section, and is deeply stained. The staining should be done in a watch-glass, which is covered up, so that the solution may not turn violet throughout during the operation.

The following were the examples tested with azo-blue in the Schizaeaceae:—

Lygodium japonicum, petiole; *L. dichotomum*, petiole; *Schizaea digitata*, rhizome; *Anemia Phyllitidis*, petiole; *Mohria caffrorum*, petiole. No callus was found in any of the specimens examined. A similar negative result was obtained in the Hymenophyllaceae. In this family the sieve-tubes were tested with azo-blue in:—*Trichomanes radicans*, rhizome and petiole; *Trichomanes Prieurii*, petiole; *Hymenophyllum demissum*, var. *nitens*, rhizome.

The results obtained with azo-blue in other Ferns may as well be mentioned. Well-marked callus occurs on the sieve-plates of the end-walls and vertical walls of the sieve-tubes in both rhizome and petiole of *Pteris aquilina*. Equally distinct callus was also found in the petiole of *Thyrsopteris elegans*, and in the rachis of *Onoclea germanica*. A small amount was

seen on some sieve-plates in the root of *Dicksonia antarctica*, and in the rhizome of *Davallia elegans* and *Nephrodium Thelypteris*. In *Angiopteris evecta* however the root and petiole showed no callus in the sieve-tubes; but in an old petiole lumps of a substance staining rather like callus occurred in many parenchymatous cells of the ground-tissue. In the petiole of *Todea barbara* no normal callus appeared to be present, but irregular masses of a substance like callus were present in some sieve-tubes and other elements. In appearance it suggested a degeneration product of the cell-wall.

It must be pointed out that where a negative result was obtained with azo-blue, the sieve-tubes of the plant may perhaps exceptionally or at some seasons produce callus. But from the nature of the results, it seems probable that callus is absent or only rarely formed in the Schizaeaceae and Hymenophyllaceae, but that its formation is general in the Polypodiaceae. No general deduction will be drawn as to the Cyatheaceae, Marattiaceae, and Osmundaceae, as only one example of each was examined.

As the sieve-tubes of the Hymenophyllaceae were only shortly described in the previous part of this paper (Boodle, '00, p. 457), a few details may be added here. In Schulze's solution the walls of the sieve-tubes of *Trichomanes radicans* stain slightly blue (distinctly bluer than the walls of the pericyclic cells), while the sieve-plates on the vertical walls are seen in surface-view as nearly colourless areas which often have several yellow-stained granules (probably of proteid) on them. The granules appear to be sometimes scattered indiscriminately in a sieve-tube, but are often distinctly grouped on the sieve-plates, and much less numerous on the parts of the wall destitute of sieve-plates. Nuclei are perhaps occasionally retained in some sieve-tubes, but are usually evidently absent, while they are constantly present in the adjacent parenchymatous cells. Fig. 47 represents two sieve-tubes and one adjacent pericyclic cell from the rhizome of *Trichomanes radicans*. Sieve-plates with granules are seen in the sieve-tubes, and there is a much elongated nucleus (*n.*) in

the pericyclic cell. Sieve-plates in section in the longitudinal walls of the sieve-tubes may be best seen when the walls are slightly swelled with Schulze's solution, as in the case of Fig. 47. After long-continued immersion in this reagent the walls become too much swelled and indistinct. Sieve-plates in section are not well seen in preparations stained with haematoxyline and mounted in canada-balsam, because the walls then become comparatively thin through dehydration, as is seen in Fig. 48. The sieve-tubes are elongated elements with very long inclined end-walls (Fig. 48, *e.*).

The conclusion to be derived from additional observations made since the publication of the first part of this paper is that the sieve-tubes of the Hymenophyllaceae agree in structure with those of other Ferns.

In examining the sieve-tubes the question of the perforation of the sieve-plates¹ was not attacked, but the observations on callus may be compared with some of the previous investigations on the sieve-tubes of Ferns.

De Bary ('77, p. 181) stated that the sieve-plates were not callous, and that he had seen the granules of contiguous sieve-tubes connected by thin filamentous processes which traverse the transverse pores in longitudinal sections of *Pteris aquilina*. Janczewski ('82, p. 89) describes callus blocking the pores of the sieve-plate in *Pteris aquilina*, but regards it as quite exceptional for the Vascular Cryptogams. Russow ('82, p. 208) states that he has met with callus generally in the Vascular Cryptogams, but only traces of it in the Marattiaceae and Ophioglosseae. He mentions that the callus often takes the form of little rods traversing the membrane of the sieve-plate, and that there are usually small brilliant granules at the extremities of the callus-rods. In *Pteris aquilina* Terletzki ('84, p. 487) describes refractive granules as aggregated on the pores of the sieve-plates, and quoting De Bary's statement with regard to the filamentous connexions between the refractive granules, remarks that the granules are not connected,

¹ Poirault ('93, p. 139) finds the sieve-plates perforated in Ophioglosseae and Marattiaceae.

but that the whole protoplasm of the sieve-tubes is connected, and represents it so, e. g. in his Fig. 26. Poirault ('93, p. 138) found callus blocking the pores of the sieve-plates generally in the Ferns, though absent in Ophioglosseae and Marattiaceae. He points out (p. 139) that Terletzki had confused under the same name ('very refractive bodies adhering strongly to the wall') the proteid granules and the callus stoppers. Poirault found that azo-violet left the former uncoloured, but stained the latter bright pink.

The results obtained with azo-blue correspond on the whole with those of Poirault, in that callus was found in *Thyrsopteris*, in *Dicksonia* and in several of the Polypodiaceae, but not in *Ophioglossum* nor in Marattiaceae. However, callus was not found in Schizaeaceae nor in Hymenophyllaceae, though these Orders are not cited by Poirault as exceptions to his generalization.

In *Pteris* and in *Thyrsopteris*, where the callus was specially well developed, it took the form of a small rod running through the pore, and often e. g. on the vertical walls expanded into a round knob at each end. The knob was frequently surrounded by a perfectly neat ring of refractive granules. This structure corresponds with Russow's description, but De Bary ('77, p. 181 and accompanying figure) did not distinguish between the refractive granules and the callus-knobs, and evidently saw the callus-rods but apparently took them for protoplasmic connexions, as Terletzki also seems to have done. Callus-rods are also found in the pits connecting sieve-tubes with phloem-parenchyma-cells.

Poirault ('93, p. 138) states that callus is formed very early on the pores of the sieve-plates, and the callus, when formed, appears to completely fill the pores; so it seems that in *Pteris aquilina* and many other Ferns the sieve-tubes have no protoplasmic connexions through the sieve-plates, except perhaps at an early stage. But there is the possibility that there may be a delicate protoplasmic thread running through the callus-rod and only recognizable by more refined methods.

FIBRES.

Fibrous elements, as described above, occur in the petiolar bundle of certain species of *Lygodium*, *Anemia*, and *Schizaea*, but are absent in *Mohria*. In all three genera they appear to represent thickened and lignified sieve-tubes. In Gwynne-Vaughan's interesting paper on *Loxsoma* ('01, p. 83, &c.) similar fibres are carefully described in that genus, and their nature in that and other cases discussed. The conclusion he arrives at is that in *Loxsoma* the fibres represent elements of the phloem originally designed for sieve-tubes, and he extends the same explanation to the fibres of *Lygodium*, *Schizaea*, and *Anemia*. The position and structure of the fibres in these three Schizaeaceous genera entirely support this view, and further in *Lygodium lanceolatum* and in *Anemia Phyllitidis* some of the fibrous elements may have even functioned as sieve-tubes prior to their sclerosis. This is suggested by the presence of granules on the end-wall of Fig. 19, and by the late sclerosis of some of the large sieve-tubes in the species of *Lygodium* just mentioned. In the case of *Trichomanes Prieurii*, as pointed out by Gwynne-Vaughan ('01, p. 86), all the sclerotic elements cannot be regarded as derived from sieve-tubes, as the end-walls of some of them do not at all resemble sieve-plates. Others however, judged by their structure, might represent sieve-tubes, as in the element shown in Fig. 46. No granules were observed on such end-walls, and it must be remarked that structures almost identical with the example figured may be met with in other sclerotic tissues, for instance in the epidermis of a rather old petiole of *Mohria caffrorum*. Hence it is necessary to depend more on the position of these elements in *T. Prieurii* for an explanation of their nature. This was chiefly relied on in the paper on the Hymenophyllaceae (Boodle, '00, p. 475), where the fibres were regarded as being derived from parenchyma. This still appears extremely probable for the bulk of the fibres, but it must be left doubtful whether some of them may have been derived from sieve-tubes. Some of the fibres lie

embedded in the xylem, and these no doubt are derived from xylem-parenchyma; some are found just outside the xylem, and a few of these are in contact with the phloem, and may perhaps represent sieve-tubes; and again among the large central mass of sclerotic elements, a few of the fibres adjacent to the incurved ends of the phloem-zone may be lignified sieve-tubes, but the majority of the internal fibres are larger elements than the sieve-tubes, and in the median upper region they correspond closely in size with the adjacent parenchyma, which lies between them and the endodermis. Gwynne-Vaughan ('01, p. 86) suggests that perhaps both sieve-tubes and parenchyma are implicated in the sclerosis. As stated above this may be the case, but, as far as present data go, the fibres may on the other hand all be parenchymatous. In that case they would be of a different nature from those of the Schizaeaceae. Outside the Schizaeaceae, Hymenophyllaceae, and Gleicheniaceae, fibres in the bundle-tissue appear to be rare among Ferns. The example given by Thomae ('86, p. 129) is that of *Adiantum trapeziforme*. Fig. 45 is a drawing of a small piece of one of the petiolar bundles. The fibres (*f.*) occur both in the xylem among the tracheides, and on the inner and outer side of the xylem band; those at the top right-hand corner of the figure being on the inner side. It is no doubt, as Gwynne-Vaughan suggests, a case of sclerosis of xylem-parenchyma. Probably few if any of the outer fibres represent sieve-tubes, so most at any rate of these sclerotic elements belong to a different category from the fibres of the Schizaeaceae. Fig. 44, which represents half the petiolar bundle of *Nephrodium Thelypteris*, is included here to illustrate the occurrence of thick-walled, unlignified elements, apparently sieve-tubes, in a member of the Polypodiaceae. They occur within the hook of the xylem. Similar elements are mentioned by Gwynne-Vaughan ('01, p. 85) in *Davallia*, &c.

SILICEOUS DEPOSITS.

The silica-nodules in *Lygodium dichotomum* described above are the most conspicuous deposits of silica to be found in the Schizaeaceae. They appear to be very similar bodies to those described by Poirault ('93) in epidermal cells of the Marattiaceae. Besides these, the only other cases of free silica-concretions within the cell hitherto recorded among the Ferns are those found in the 'Deckzellen' of Hymenophyllaceae, and according to a statement of Mettenius ('64, p. 426), in epidermal 'Deckzellen' of the leaf of *Aspidium deltoideum*. The siliceous bodies in *Lygodium* agree with those of the Marattiaceae, but differ from those of the Hymenophyllaceae in having an indefinite form, and they differ from those of both these Orders in occurring in deep-seated tissues, e.g. in the stele.

Among the Angiosperms intracellular silica-concretions are found in the xylem-parenchyma and other tissues of *Moquilea* (Kohl, '89, p. 248), but a closer agreement with *Lygodium* as to the form of the silica-nodules is found in the Podostemaceae.

According to Kohl, Ferns are mostly poor in silica (e.g. about 5 per cent. of the ash), but *Blechnum Spicant* and *Pteris aquilina* are cited as having a large amount (about 53 and 45 per cent. respectively). The silica in these cases is possibly an infiltration of the cell-walls of certain specialized epidermal cells, as described by Kohl in *Antrophyum*, &c.

A few silica-nodules similar to those of *Lygodium* were found in the ground-tissue of *Anemia*, but none in *Schizaea* or *Mohria*.

In *Schizaea digitata* and in *Anemia Phyllitidis* the outer wall of the epidermis of the petiole has small warts arranged in longitudinal rows. Each is due to a refractive, probably siliceous body embedded in the wall. They have been described by Prantl, and figured by him ('81, p. 33, and Taf. 4, Fig. 50), in *S. pennula*, Sw., and by Britton and Taylor ('01, Pl. 6, Fig. 93) in *S. pusilla*, Pursh.

HAIRS.

The hair-structures of the Schizaeaceae need not be fully described here, as details are given by Prantl ('81, p. 35). Two kinds of hairs are found, namely stout or long hairs, each consisting of a single row of cells, which may or may not bear a glandular cell at the top, and short glandular hairs, consisting of either one or two cells, and usually adpressed. The glandular cell of these short hairs is therefore either seated on the epidermis or on a single stalk-cell. It is oval, and has a distinct nucleus. The larger kind of hairs, in this case blackish and not glandular, are found for instance densely covering the stem apex and leaf-rudiments of *Lygodium dichotomum*. On the young petiole of *Anemia Phyllitidis* there are both long filiform brown hairs (e.g. 3 mm. in length), and small unicellular glandular hairs. Both kinds of hairs of similar proportions were found by Britton and Taylor ('01, p. 13) in *Schizaea pusilla*.

Mohria caffrorum is exceptional in possessing paleae, resembling those of the Polypodiaceae, but it is interesting to find that in *Lygodium dichotomum* longitudinal divisions occasionally take place in the long hairs, so that the basal part is converted into a flat plate of cells, i. e. a small palea. Prantl does not appear to have seen any such cases, but he regarded the long uniseriate hairs as homologous with the paleae of other Ferns ('81, p. 37). The rhizome of *Mohria* bears paleae, long rhizoid-like hairs, and also very numerous unicellular glandular hairs, identical with those of *Anemia*.

THE STELE.

An important paper dealing with the stele has recently been published by Jeffrey ('00). In this paper the structure of the stem in seedling plants belonging to several groups has been carefully described, and the manner in which the mature structure is attained has been followed, the changes in the disposition of the vascular elements and the behaviour of the endodermis being worked out in relation to the leaf-gaps. Further, wide generalizations as to the morphology

of stelar structures are arrived at. One of these is that Van Tieghem's three types of central cylinder are modifications of the siphonostelic type, i. e. a vascular tube with leaf-gaps. Basing one's terminology on the position of the endodermis, this is evidently the case for the examples investigated, and is probably generally true for the polystelic type. But it must be recognized that the homology of different types of stelar structure is a difficult question, and is not at once disposed of by merely tracing the continuity of the various tissues. Perhaps in the present state of our knowledge it does not admit of any certain solution.

One would be inclined to describe Fig. 31 as a horseshoe-shaped stele with a projection of ground-tissue in its concavity, and to regard the endodermis as limiting the ground-tissue towards the stele. This is no doubt a convenient description, but there is no proof that it is anything more than a physiological use of terms, which does not necessarily agree with the morphology of the parts. The central 'ground-tissue' is certainly continuous with, and similar to, the external ground-tissue, but one must regard it as possible that in this case the stele may really have a roundish outline, and that part of its tissue has been differentiated as parenchyma with the characters of ground-tissue, and limited by a piece of endodermis formed from stelar tissue so as to join the external endodermis, which is interrupted opposite the stelar parenchyma. The endodermis is usually formed from the innermost layer of the cortex¹, but that the endodermis is not dependable as a morphological limit in certain other cases is sufficiently shown by the species of *Equisetum*² as pointed

¹ In several Ferns at any rate the pericycle is formed by subdivision of the same layer, as described by Van Tieghem ('88, p. 404) for the stem of *Hymenophyllum* and the stolon of *Nephrolepis*, and the same is probably the case in *Lygodium*. Other cases are quoted by Strasburger ('91, p. 446), but these being polystelic involve other questions.

² The parenchyma-rays between the bundles would have a different morphological value in nearly allied species (gamodesmic and dialydesmic), if the endodermis were regarded as always limiting stelar tissue. Jeffrey ('99, p. 157) regards Strasburger's conclusions as invalidated by the structural facts cited by Van Tieghem ('90), but the case just mentioned appears to weigh heavily against the morphological value of the endodermis.

out by Strasburger ('91, p. 441). On account of his view of the different morphological nature of the endodermis in different cases, Strasburger used the term 'endodermis' in the physiological sense, for the layer bearing cuticularized bands, and introduced the term 'phloeoterma' to designate the innermost layer of the cortex ('91, p. 435); but he used the term phloeoterma in a way that may be called in question in the case of polystelic Ferns. As the morphological value of the endodermis cannot be said to have been definitely established in the case of 'polystelic' plants, it would be preferable to call the cutinized layer in them an endodermis, and not to apply the term phloeoterma to it, as Jeffrey and Gwynne-Vaughan have done, until it has been if possible proved that this layer *is* the innermost layer of the cortex. In Jeffrey's work the endodermis is regarded as revealing the position of the limit of the cortex, and is called phloeoterma; and the continuity of tissues in the seedling is relied on for determining their morphological nature.

The latter point is shown by the following statement regarding *Ranunculus*:—'In the young axis the stelar system possesses an internal phloeoterma, which is continuous with the external phloeoterma through the foliar gaps, and is therefore of the same morphological value.' This conclusion cannot be said to carry conviction. It is not wished in any way to imply the impossibility of such interpretations being correct, but simply to point out that proof is required as to the morphological nature of the endodermis, and as to the value of the continuity of tissues.

In relation to the questions involved it will be as well to attempt to regard some of the structures from a physiological standpoint. The function of the endodermis appears to be to form a closed layer of cells shutting off vascular tissue from tissues which contain intercellular spaces (Strasburger, '91, p. 611), and thus to prevent air from penetrating to the tracheae. The mechanical firmness which enables the endodermis to escape rupture of its cells is attained by the network formed by the tangential suberized bands (Haberlandt, '84,

p. 245). In the stems of many Monocotyledons a function similar to that of the endodermis is probably performed by the sclerotic sheaths of the bundles, which would prevent accidental ruptures in the ground-tissue from continuing the intercellular spaces right up to the tracheae. In Dicotyledons, where intercellular spaces occur in the pith, the perimedullary zone may perhaps have a similar function. But the spoiling of a few tracheides would be comparatively unimportant in plants which form new ones by secondary thickening. In the Ferns, on the other hand, the endodermis seems to be an important layer, though often assisted in its function in the mature plant by adjacent sclerotic tissue. The vascular tissue is therefore normally completely enclosed by an endodermis, especially towards any loose parenchyma. In solenostelic forms the connexion of inner and outer endodermis at the leaf-gap is of course necessary from this point of view, if a leaf-gap is to be formed. We will now assume the hypothesis mentioned previously, that the horseshoe-shaped 'stele' is morphologically only part of a circular or oval stele, and further that in the polystelic (or dialystelic) type the limit of the stele is also circular and includes the separate 'steles' and the central ground-tissue. The following might then be a physiological description of what has taken place. The primitive solid stele has become much enlarged by increased cell-division in the cylinder of cells which were set apart to produce it. This increase in diameter may be connected with the attachment of the tangentially broad leaf-traces of large leaves¹, especially if they are polystichous. A *solid* mass of tracheides of the increased diameter is not necessary for the water-supply, and consequently the central cells do not attain to tracheide-formation, but remain parenchymatous or become sclerotic as the case may be. At this stage one would have a ring of xylem surrounded by phloem, and enclosing a central pith. This is found in *Schizaea*. In *S. digitata* the pith is

¹ Jeffrey ('00, p. 38) connects the siphonostelic type of structure with mechanical strengthening of the axis to enable it to bear large leaves; this may be one factor, but sclerotic tissue is the *chief* supporting tissue in Ferns.

sclerotic, in *S. fistulosa* it is parenchymatous (or collenchymatous). There is no internal endodermis in these species; which is perhaps connected with the fact that there are no intercellular spaces in the pith. In the next stage in complication, internal phloem is present, differentiated from tissue near the inner limit of the xylem. This would be formed for the attachment of the inner phloem of a concentric or bicollateral petiolar bundle. *Schizaea* having collateral petiolar bundles has no internal phloem in the stem. The type with internal phloem is not necessarily derived from the medullated type with none. If the petiole was already concentric at the time when the solid type of stele was departed from, internal phloem might be formed as soon as there was any centrally placed soft tissue. The one or the other structure might appear first in different phyla. No mature Fern appears to be known with internal phloem, and at the same time no inner endodermis, but this condition is met with in the seedling stem of *Anemia*. This is where there is not much tissue within the phloem; but, when a large mass of parenchyma with intercellular spaces is present, an inner endodermis becomes necessary. This is the solenostelic stage. This central tissue is not required by the stele, and for it to be of use, e.g. in storing up starch, it must be in free connexion with the outer ground-tissue; hence (as all the walls of endodermal cells frequently become suberized) the necessity for leaf-gaps as found in the solenostele. Continuity being established between the central and outer ground-tissue, they may have the same functions and resemble one another structurally. Nothing further need be said as to the dialystelic type, which only differs from the solenostelic in its more crowded leaf-gaps.

The preceding is a purely theoretical discussion, and of course proves nothing as to the correctness of the hypothesis with which we started. One may now see whether anything can be done towards solving the morphological problem. The structure of the seedling is accepted as giving some clue to the course of phylogenetic changes. In describing the seedling of *Pteris aquilina*, Jeffrey ('00, p. 9) points out

that there is no evidence for the repeated bifurcation of the young central cylinder, described by Van Tieghem as characteristic of his polystelic type. The structure of the seedling of *Anemia* bears this out; the stele as a whole does not appear to branch. In *Anemia*, internal phloem appears, when there are only a few soft elements inside the xylem. This may mean that a medullated stage was not passed through in this particular phylum, or it may be a case of the early appearance of an acquired structural character. Again, in the seedling solenostelic structure precedes dialystelic, which may have phylogenetic significance. But these facts do not elucidate the morphological difficulties. Neither does investigation of the apex greatly help the matter. Gwynne-Vaughan ('97, p. 323) found in species of *Primula*, with several steles arranged in a slightly interrupted ring, that these were represented in the apex by a similar ring of desmogen-strands, which could be followed up as far as any differentiation could be distinguished in the meristem. This practically describes what was found in the apical region of an oldish seedling of *Anemia Phyllitidis*. This was to be expected. When differentiation begins in a meristem, the form and mode of division of its elements is intimately connected with the form and arrangement of the tissue-elements to be produced, so one is not likely to learn much from these characters in the meristem, that cannot be seen in the mature structure. The relation of the meristematic tissues to the divisions of the segments of the apical cell could not be made out. If this could be determined, it would carry some morphological weight, especially in nearly allied forms. If, for instance, it could be shown that the steles of *Anemia Phyllitidis* together with the central ground-tissue were formed from similar divisions of the apical cell-segments to those from which the solid stele of *Lygodium* is formed, a presumption of morphological identity would be established. The arrangement of cell-walls in the apical region of *Anemia* did not suggest what might be called an *intrusion* of cortex into the stelar ring.

An important method of investigation is a comparison of the structure in nearly allied species. A thorough examination of several species of *Anemia* would be most interesting from this point of view, but sufficient material has so far not been obtainable. One or two points that have some bearing on the endodermis and stele, however, must be mentioned. *A. mexicana* is solenostelic with a central mass of sclerotic tissue inside the inner endodermis. This is homologous with the central ground-tissue of *Anemia Phyllitidis*. Such tissue may become sclerotic in polystelic forms, as seen in *Mohria*. The formation of abundant sclerotic tissue is rather a character of xerophytic forms, and its function, besides being mechanical, is possibly connected with the storage of water in its walls and cavities. *A. mexicana* is distinctly xerophytic in appearance, which would be expected, as it is described as growing on rocks. In this species at the node a group of sclerotic tissue appears to become detached from the central sclerotic ground-tissue and to pass out behind the leaf-trace so as to join the external sclerotic ground-tissue, as in *Mohria*; there is thus a leaf-gap in the endodermis. In the node of *A. coriacea*, which is probably nearly related to *A. mexicana*, no sclerotic tissue passes out, and the endodermis is not interrupted, hence, judged by the node examined, this species is not properly solenostelic. If this character were constant for all the nodes of *A. coriacea*, its central ground-tissue, judged by continuity, would have a totally different morphological nature from that in *A. mexicana*; which is extremely improbable. This matter must be left for future investigation, but it may be pointed out, that from the physiological point of view the connexion at the nodes between the inner and outer ground-tissue, when they become sclerotic, is comparatively unimportant, except perhaps that an occasional connexion might be necessary, either as a tie between two mechanical systems, or for the slow transference of water from one to the other.

Another point suggested by these species is that, assuming them to have been derived from polystichous forms by

suppression of the leaves on the lower side of the creeping rhizome, which at any rate appears probable, the parenchymatous tissue representing the original leaf-gaps on the lower side appears to have been converted into vascular tissue, and the leaf-gaps thus repaired. This would agree with the view that the leaf-gap-parenchyma is really stelar tissue in the phylogenetic sense. Again, *A. aurita*, if its structure were better known, would form an interesting type for comparison with *A. mexicana*.

What has been said above may at any rate show that a fuller investigation of species of *Anemia* would probably throw a great deal of light on questions connected with the morphology of the stele.

A few more remarks must be made with regard to the seedling. There is probably a tendency for plants to show acquired structural characters at earlier and earlier stages in their ontogeny. The strip of endodermis (*e.*) seen in Fig. 28 is apparently useless, and may represent an attempt to form a loop of endodermis such as is found at a later leaf-gap (Fig. 31), and for which in the small stele there is not sufficient room. The early connexion between inner and outer phloem and between other tissues in the seedling suggests a possibility, though this is very hypothetical, that when a new kind of tissue is to be formed, e. g. internal phloem from stelar parenchyma, contact with similar tissue, in this case phloem, may be necessary. That is to say, protoplasmic continuity between different elements when young may in some way help in leading to the formation of corresponding mature elements; or one might say a 'contagion' for forming a special kind of element is handed on.

To conclude the subject, the homologies of different types of stelar structure at present appear doubtful, but the writer inclines to the view that the medullated, solenostelic, and dialystelic types have been derived from the solid stele by the transformation of its central tissues into parenchyma in the first case, and into parenchyma together with a certain amount of phloem and endodermis in the second and third cases.

The time for the application of rigorous terminology does

not seem to have arrived, but 'dialystelic' and 'solenostelic' are convenient words for describing structures like those of *Anemia Phyllitidis* and *A. mexicana*, whether they are really based on the disposition of the whole stelar tissue, or of the part of the stele which shows typical stelar characters. The separate parts of the dialystelic type have here been usually spoken of as 'steles'; but 'meristeles' or 'dialysteles' would be perhaps a preferable term, if one regards them as being only circumscribed parts of a stele.

COMPARISON OF STRUCTURE.

Some points of agreement and difference between the structure of the genera of Schizaeaceae should now be referred to.

A *single* leaf-trace passes off to supply each leaf, and is continued as the petiolar bundle which remains undivided. In the bundle of the petiole (of three of the genera) a median protoxylem-group is present on the upper side of the xylem. Two other protoxylem-groups, which are also on the upper side of the xylem, may also be present (e.g. *Anemia*). In *Lygodium*, on the other hand, two approximated groups of protoxylem separated by the median plane are present on the *lower* side of the xylem. These, however, tend to fuse into one median group towards the base of the petiole, and in *L. palmatum* one median group is found in the petiole. In all the species of *Lygodium*, besides the protoxylems mentioned, two lateral external groups are also present. The two nearly median protoxylem-groups have probably been produced by the splitting of a median single group, as found near the base of the petiole, for that is probably a less modified region. Therefore *L. palmatum*, with its undivided median protoxylem, shows a less modified structure than the other species, and it is noteworthy that in this species the median group is not peripheral but embedded in the xylem. A possible explanation of the great difference in type between the bundle of *Lygodium* (Fig. 4) and that of *Anemia* (Fig. 17), is that in *Lygodium* there has been a large addition of xylem

elements in the region corresponding with the concavity of the xylem-arch in *Anemia*, and a suppression of the xylem external to the three protoxylem-groups, except in the case of the median group in *L. palmatum*, together with a splitting of the median group except in *L. palmatum*. In both *Anemia* and *Lygodium* the median group (or pair of groups) differentiates before the others. In *Schizaea* only the median endarch group appears to be present. Possibly one median endarch protoxylem-group is primitive for the Order, and the lateral groups have been added in connexion with increase in size of the bundle. The protoxylem-groups in the petioles of all the genera contain spiral or annular elements, but in *Lygodium* and *Schizaea* spiral elements are absent in the basal region of the petiole; but in *Lygodium* at any rate small early-formed scalariform tracheides occur in a nearly identical position to that occupied by the spiral elements higher up. The term 'protoxylem' appears applicable to these. In the Schizaeaceae the stem-protoxylem does not occur in definite groups, but is represented by scattered tracheides which are scalariform. No spiral tracheides are present in the stem except in *Anemia* and *Mohria*, where a spiral protoxylem of the leaf-trace is continued a short distance down in the stem. Gwynne-Vaughan ('01, p. 87) has laid stress on the distinction between stem- and leaf-protoxylem in *Loxsonia*; and it certainly appears probable that the differentiation of stem-protoxylems in close relation to leaf-trace-protoxylems may be a comparatively late character. Where the xylem is of considerable thickness the protoxylem-elements of the stem are at or near the periphery.

Sclerosed sieve-tubes, forming fibres in the petiolar bundle, occur in certain species of *Lygodium*, *Schizaea*, and *Anemia*, but not in *Mohria*.

Fibres of this kind are uncommon among Ferns. Their presence here, and the occurrence of 1-2-celled glandular hairs on the stem and petiole, and of silica-deposits, within the cell-cavity (in *Lygodium*), or in the form of knobs in the outer walls of epidermal cells (in *Schizaea* and *Anemia*) may

be pointed out as rather characteristic features in the Schizaeaceae.

Four types of stele are represented among the Schizaeaceae:—the solid stele in *Lygodium*, the medullated stele in *Schizaea*, solenostely in some species of *Anemia*, and dialystely in other species of *Anemia* and in *Mohria*. With regard to *Schizaea*, as the transitional region of seedlings has not yet been specially examined, it cannot be said whether any different type of structure occurs at an early stage in the ontogeny, as seen by Jeffrey in some Angiosperms. De Bary ('77, p. 344) agrees with Russow's view that the central tissue within the xylem of *Schizaea* is not to be regarded as a pith, but as parenchyma belonging to the stele. But as pith is not a sharply characterized term there is no harm in applying it to this tissue, especially if one inclines to the view that phylogenetically pith is stelar tissue. A structure is found in *Anemia coriacea*, which though resembling solenostely may differ in the behaviour of the endodermis at the node.

The petiole-bundle is concentric in *Lygodium*, collateral in *Schizaea*, and roughly speaking bicollateral in *Anemia* and *Mohria*.

The structure of *Anemia* and *Mohria* must be compared, as Christ ('97, p. 352) regards the external characters of the latter as so anomalous for the Order. *Mohria* resembles *Anemia Phyllitidis* completely in the type of its stelar system, and agrees roughly with other species of *Anemia*, as to the dimensions of the parts. The petiolar bundle in *Mohria* is of the type found in *Anemia*. There are no fibres in the bundle of *Mohria*, but that is also true for certain species of *Anemia*, e.g. *A. Dregeana*. *Mohria* possesses glandular hairs exactly similar to those of *Anemia*, but differs in possessing paleae. An approach to palea-formation occurs however in *Lygodium*, therefore this character is not of great importance. Again, the sclerotic group accompanying the petiole-bundle of *Mohria* is suggested by the thick-walled elements which occur in a corresponding position with regard to the bundle at the base of the petiole of *Anemia Phyllitidis*.

Finally the sclerotic central ground-tissue in the stem of *Mohria* finds its counterpart in the solenostelic *A. mexicana*. The structure of *Mohria* thus suggests that the genus is probably quite nearly allied to *Anemia*, but it shows some characters which suggest greater tendency to xerophytism, than is found in *A. Phyllitidis*.

Judged structurally, *Anemia* and *Mohria* are less primitive than *Lygodium* and *Schizaea*; and of these *Lygodium*, having a solid stele, should be the more primitive. The petiolar bundle of *Lygodium*, however, is concentric, which is probably not a primitive character. It must be remembered that the well-developed leaves of this genus are specialized for twining, and it is probably because of this, that the bundle forms a rounded cylinder instead of conforming more to the type found in the other genera. The form of the petiole-bundle may in its turn have determined the retention of the primitive type of stele, because a solid cylindrical stele would be suitable for the attachment of a similarly shaped leaf-trace. Hence the structure of the stem alone should perhaps not be taken as pointing to the genus being necessarily more primitive than *Schizaea*. It is interesting, however, to observe that Bower ('99, p. 44) found that among the Schizaeaceae *Lygodium* showed the largest sporangia and the largest output of spores, and that these characters generally indicate primitive nature.

A comparison of the structure of the Schizaeaceae with that of the Hymenophyllaceae and of other Ferns will be reserved for the concluding part of this paper, on the Gleicheniaceae, but one comparison may be made here.

The structure of some species of *Anemia* shows considerable resemblance to *Loxsoma*. Gwynne-Vaughan ('01, p. 94) regards these resemblances as insufficient to constitute a *close* relationship, and this appears to be the case; but perhaps a closer relationship to the Schizaeaceae than to the Hymenophyllaceae is suggested. The facts may be stated thus:—the solenostele of *Loxsoma* resembles that of *A. mexicana* in the elements of the stem-protaxylem being scalariform and scattered, and also approximately in their position. The

possession of sclerosed sieve-tubes is common to both genera, and there is a single petiolar bundle in both, of a somewhat similar form, and it is endarch. On the other hand, *Loxsoma* has more numerous protoxylem-groups, and there is no median group. At the top of the petiole, however, there are only four groups, two in the hooks of the xylem, about as in *Anemia*, and two at the ends of the top of the arch. These two may have originally been produced by splitting of a median group, as has been supposed in the case of *Lygodium*. The distribution of the fibres in *Loxsoma* is different from what occurs in *Anemia*; they extend nearly all round the xylem. Another point of difference is that in *Anemia* the spiral elements of the leaf-trace are continued a short way down the stem. Further, the only solenostelic forms in *Anemia* are probably to be regarded as reduced from dialystelic forms; and these latter would show less resemblance to *Loxsoma* in stem-structure, than the species chosen for comparison; though this is perhaps less important.

REFERENCE TO LITERATURE.

A few further allusions to Prantl's work on the Schizaeaceae and to that of one or two other authors ought to be made. A full description of the form and veining of the leaves, and of the fructifications, is given by him. He also gives a detailed account of the vegetative structure, which is to a great extent correct. He recognized that the distinction between the solenostelic and dialystelic species of *Anemia* is one of degree, and is related to the phyllotaxy (p. 22). Only a few special points in his account of the anatomy need be referred to. In *Schizaea elegans* some large reticulate tracheides on the median lower side of the petiole-bundle are described as being the first to differentiate (p. 24). If this is correct, it is different from *S. digitata*. The petiole of *Anemia Phyllitidis* is well described and figured. In the petiole of *A. coriacea* a curious bundle-structure is described, in which the xylem is separated into two bands. In the stem of *Lygodium* the protoxylem is stated to be 'regellos über

den Strangquerschnitt zerstreut,' but this is not correct. The protoxylem elements of the stem were recognized by Prantl as scalariform in *Schizaea*, but for the other genera he describes them as reticulate, which has not been confirmed. Prantl records the agreement of *Mohria* with most *Anemias* in the structure of its bundles.

Bauke ('78, p. 642) shows that *Anemia* and *Mohria* exhibit a special type of development in their prothalli, distinct from that of the Polypodiaceae, and points out that this resemblance is of importance, as these two genera are unlike in habit. This indication of affinity fits in well with the anatomy.

Russow ('72) gives a short account of the anatomy of the Schizaeaceae (p. 97). In the stem of *Lygodium* he refers to the irregular order of development of the tracheides. He also describes the fibres in the petioles of *Schizaea* and *Anemia*. The petiole-bundle of *Anemia* is fairly correctly represented in his diagram (Pl. X, Fig. 9), except that the protophloem is drawn as continuous. In *Schizaea pectinata* also (Pl. X, Fig. 7), the protophloem is made to surround the xylem.

Thomae ('86, p. 155) quotes several of Prantl's statements with regard to the Schizaeaceae and does not add many new observations.

Britton and Taylor have given an account of the life-history of *Schizaea pusilla*¹. The structure of stem and petiole as described by these authors agrees in type with *S. digitata*.

SUMMARY OF SOME OF THE RESULTS.

1. The sieve-tubes of the Schizaeaceae and Hymenophyllaceae do not appear to form callus, but in other respects (perhaps excepting *Schizaea*) agree structurally with the sieve-tubes of other Ferns.

2. Silica-nodules occur in the cavities of parenchyma-cells in some species of *Lygodium* and *Anemia*.

3. The fibres in the petiolar bundle of some species of

¹ I am indebted to Mrs. Britton for material of this species, but have not yet made any preparations of it.

Lygodium, *Schizaea*, and *Anemia* are to be regarded as modified sieve-tubes. The fibres in *Trichomanes Prieurii* appear to be formed, either all from parenchyma, or some from parenchyma and some from sieve-tubes.

4. The species of *Lygodium* are very uniform in structure, and possess a solid stele; *Schizaea* has a medullated stele, while dialystelic structure is found in *Mohria* and some species of *Anemia*, solenostelic structure in others.

5. The seedling of *Lygodium* does not suggest reduction from a more complicated type.

6. In the seedling of *Anemia* the solid stele is converted into a dialystelic one, by gradations similar in type to those found in *Pteris* for instance; but no very definite morphological conclusions should be drawn from this fact.

7. The protoxylem belonging to the stem in the Schizaeaceae is scattered, does not include spiral elements, and is differentiated mostly with no relation to the leaf-traces.

8. The petiole possesses a single bundle.

9. The roots appear to be diarch throughout.

In conclusion it may be said that anatomically the Schizaeaceae form a most interesting group, and that further examination of some of the forms may give useful data, on which to base conclusions with regard to the homologies of the different types of stelar structure found in the Order.

I wish to express my thanks to Dr. D. H. Scott, F.R.S., for valuable advice and suggestions, and to Mr. J. G. Baker, F.R.S., and to Mr. C. H. Wright, A.L.S., for assistance as to the specific identity of some specimens. The material examined in *Mohria* and in some specimens of *Lygodium* and *Anemia* was from plants in cultivation in the Royal Gardens, Kew; in some other species dried material was used. I am indebted to Prof. J. B. Farmer, F.R.S., Mr. A. C. Seward, F.R.S., to Dr. J. C. Willis, and Mr. J. B. Carruthers of Peradeniya, and to Mrs. Britton of New York for kindly presenting me with material of species of *Schizaea*, &c.; and to Messrs. Hill of Edmonton for seedlings of *Anemia Phyllitidis*.

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EXPLANATION OF FIGURES IN PLATES XIX, XX, AND XXI.

Illustrating Mr. Boodle's paper on the Anatomy of the Schizaeaceae.

The following lettering is used in several of the illustrations: *e.*, endodermis; *x.*, xylem; *px.*, protoxylem; *ph.*, phloem; *pph.*, protophloem. Where *x'*, *px'*, *ph'*, *pph'* are used, they refer to the xylem, protoxylem, phloem, and protophloem respectively of a petiole or leaf-trace. *i. e.*, inner endodermis; *i. ph.*, inner phloem. Figs. 1, 4, and 17 are reproduced from photographs by Dr. E. C. Bousfield; Figs. 11, 38, and 44 are also from photographs.

Fig. 1. Transverse section of rhizome of *Lygodium dichotomum*. The different layers may be easily recognized by comparison with Fig. 2. × 50.

Fig. 2. Part of transverse section of rhizome of *Lygodium dichotomum*. *s. l.*, layer of suberized cells; *x. p.*, one of the xylem-parenchyma-cells; *si.*, silica-nodule in a xylem-parenchyma-cell. × 390.

Fig. 3. Part of transverse section of young rhizome of *Lygodium dichotomum*. × 390.

Fig. 4. Transverse section of petiole of *Lygodium japonicum*. × 75.

Fig. 5. Silica-nodule from xylem-parenchyma of rhizome of *Lygodium dichotomum*. × 900.

Fig. 6. Tracheide isolated by maceration from node of *Lygodium dichotomum*. × 90.

Fig. 7. Part of a small sieve-tube in the rhizome of *Lygodium dichotomum*, showing sieve-plates and granules. × 900.

Fig. 8. Section of seedling of *Lygodium japonicum*. *f.*, foot; *p.*, prothallus; *x.*, diarch xylem of primary root. × 45.

Fig. 9. Transverse section of seedling-stem of *Lygodium japonicum*, below first leaf. *p.*, one of the xylem-parenchyma-cells. × 390.

Fig. 10. *Lygodium japonicum*. First leaf-trace of the seedling, separating from the stele. × 390.

Fig. 11. *Schizaea dichotoma*. Transverse section through a node. × about 30.

Fig. 12. *Schizaea digitata*. Transverse section of young stem at node. *a.*, *b.*, examples of outer and inner tracheide respectively developing first; *m.*, pith. × 90.

Fig. 13. *Schizaea digitata*. Transverse section of petiole near base. *f.*, fibre. × 260.

- Fig. 14. *Schizaea digitata*. Transverse section of young petiole. $\times 260$.
- Fig. 15. *Schizaea dichotoma*. Diagram of transverse section of node. $\times 30$.
- Fig. 16. *Anemia Phyllitidis*. Diagram of transverse section of stem. *l t.*, leaf-trace; *r.*, roots. $\times 7\frac{1}{2}$.
- Fig. 17. *Anemia Phyllitidis*. Transverse section of petiole. $\times 75$.
- Fig. 18. *Anemia Phyllitidis*. 'Cavity-parenchyma' connected with the median protoxylem-group of the petiole. *r.*, rings of an annular tracheide. $\times 390$.
- Fig. 19. *Anemia Phyllitidis*. Fibre representing a lignified sieve-tube, from the petiole. $\times 900$.
- Fig. 20. *Anemia Phyllitidis*. Silica-nodule from the ground-tissue close outside the endodermis of the petiole. $\times 900$.
- Fig. 21. *Anemia Phyllitidis*. Transverse section of primary root of seedling. $\times 390$.
- Figs. 22 and 24–31, represent transverse sections of the seedling-stem at successively higher levels.
- Fig. 22. *Anemia Phyllitidis*. Transverse section of stem of seedling. *x'*, xylem of first leaf-trace. $\times 390$.
- Fig. 23. *Anemia Phyllitidis*, seedling. Xylem of first leaf-trace in cortex of stem. $\times 900$.
- Fig. 24. *Anemia Phyllitidis*. Xylem of stele after departure of first leaf-trace. One parenchyma-cell is seen inside the xylem-mass. $\times 390$.
- Fig. 25. *Anemia Phyllitidis*, seedling. The endodermal cells are marked with crosses. $\times 390$.
- Fig. 26. *Anemia Phyllitidis*, seedling. The four elements in the centre marked with crosses are sieve-tubes. $\times 390$.
- Fig. 27. *Anemia Phyllitidis*, seedling. *ph.*, internal phloem. $\times 390$.
- Fig. 28. *Anemia Phyllitidis*, seedling. Diagram showing a row of endodermal cells (*e.*) connected with the outer endodermis. $\times 90$.
- Fig. 29. *Anemia Phyllitidis*, seedling. Diagram showing a ring of inner endodermal cells (*e.*) enclosing one parenchymatous cell. $\times 90$.
- Fig. 30. Drawing of the same. *e.*, inner endodermis; *ph.*, inner phloem. $\times 390$.
- Fig. 31. *Anemia Phyllitidis*, seedling. Diagram showing both xylem and endodermis horseshoe-shaped. $\times 90$.
- Fig. 32. *Anemia Phyllitidis*, seedling. Transverse section of petiolar bundle of early leaf. *f.*, probably fibres. $\times 390$.
- Fig. 33. *Anemia Phyllitidis*, seedling. Transverse section of young petiolar bundle of later leaf. $\times 390$.
- Fig. 34. *Anemia Phyllitidis*, seedling. Transverse section of one of the steles in the stem; early stage with only a few tracheides differentiated. $\times 390$.
- Fig. 35. *Mohria caffrorum*. Diagram of transverse section of stem. *r.*, roots; *a.*, 'stem-steles'; *b.*, leaf-trace; *sc.*, sclerenchyma-group accompanying leaf-trace. $\times 7\frac{1}{2}$.
- Fig. 36. *Mohria caffrorum*. Diagram of transverse section of petiolar bundle. *sc.*, sclerotic cells; the phloem is shaded. $\times 45$.
- Fig. 37. *Mohria caffrorum*. Part of longitudinal section of young stem, showing two sieve-tubes (*ph.*) and one protoxylem element. $\times 390$.
- Fig. 38. *Anemia mexicana*. Transverse section of the stele in the rhizome. $\times 40$.

Fig. 39. *Anemia mexicana*. Part of transverse section of stele. *i. ph.*, inner phloem. $\times 140$.

Fig. 40. *Anemia Phyllitidis*. Part of transverse section of one of the steles in the stem. *i. e.*, inner endodermis; *i. ph.*, inner phloem. $\times 140$.

Fig. 41. *Anemia coriacea*. Diagram of stele of rhizome, just below node. *e'*, group of endodermal cells. $\times 90$.

Fig. 42. *Anemia coriacea*. Diagram of stele at node. $\times 90$.

Fig. 43. *Anemia coriacea*. Diagram of node. *l. t.*, leaf-trace. \times about 75.

Fig. 44. *Nephrodium Thelypteris*. Half petiolar bundle, showing thick-walled sieve-tubes. $\times 40$.

Fig. 45. *Adiantum trapeziforme*. Part of petiolar bundle. *f.*, fibres. $\times 390$.

Fig. 46. *Trichomanes Pricurii*. Fibre in bundle of petiole. $\times 390$.

Fig. 47. *Trichomanes radicans*. Two sieve-tubes and one pericyclic cell from rhizome. *s.*, sieve-plates; *n.*, nucleus of parenchyma-cell. $\times 390$.

Fig. 48. *Trichomanes radicans*. Three sieve-tubes from rhizome. *s.*, sieve-plate; *e.*, end-wall. $\times 390$.

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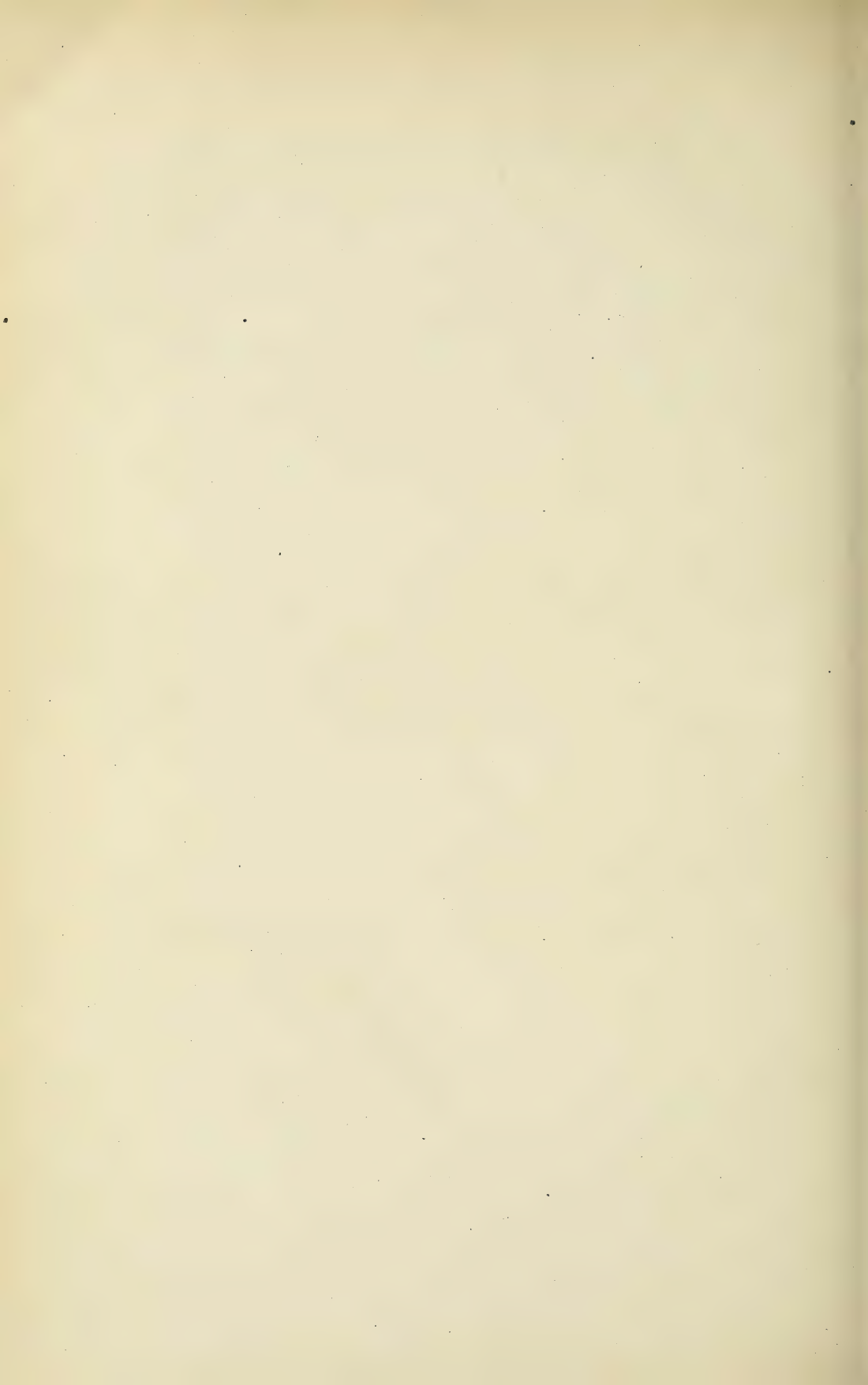
In the lower part of the petiole of *Anemia coriacea* the bundle is of the type found in other species of *Anemia*, e.g. *A. aurita*, but in the upper part of the petiole it divides into two, one bundle having a V-shaped xylem, and the other, which is smaller, having a straight band of xylem. Prantl's description ('81, p. 26) of the petiole of this species probably refers to the region where the division of the bundle is incomplete. The division of the petiolar bundle appears to be an unusually early separation of a bundle for one of the first pinnae of the leaf.

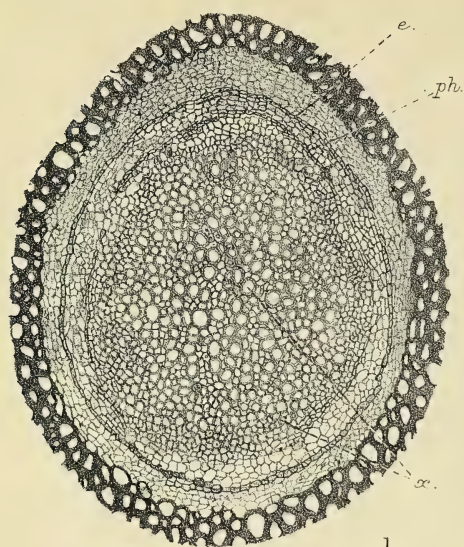
Two papers not cited in the list of Literature must be mentioned here, viz.:—

SCOTT, '94: Recent work on the Morphology of tissues in the Higher Plants. Science Progress, vol. i, and

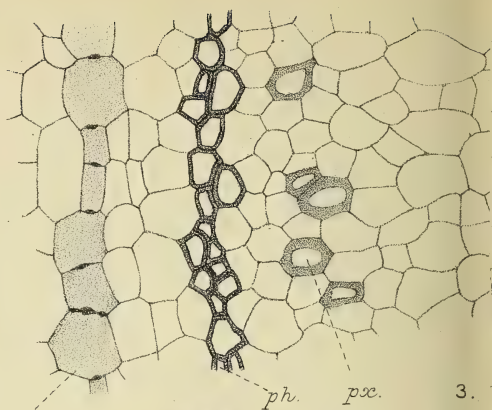
TANSLEY, '96: The Stellar Theory. Science Progress, vol. v.

In these papers several views are discussed or brought forward, which have been adopted in the present paper (see the section on the Stele, p. 403).

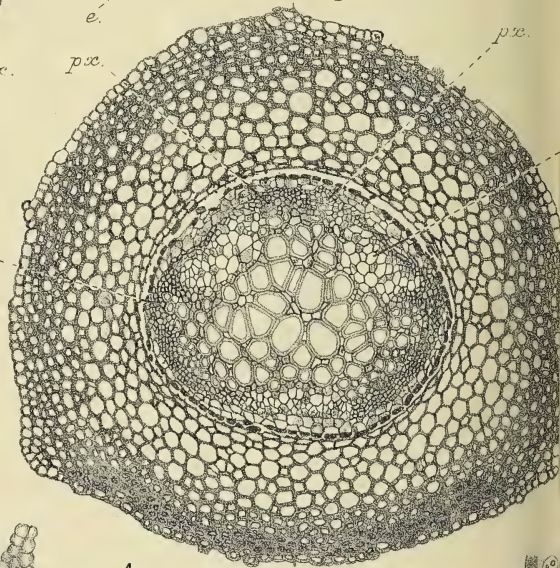




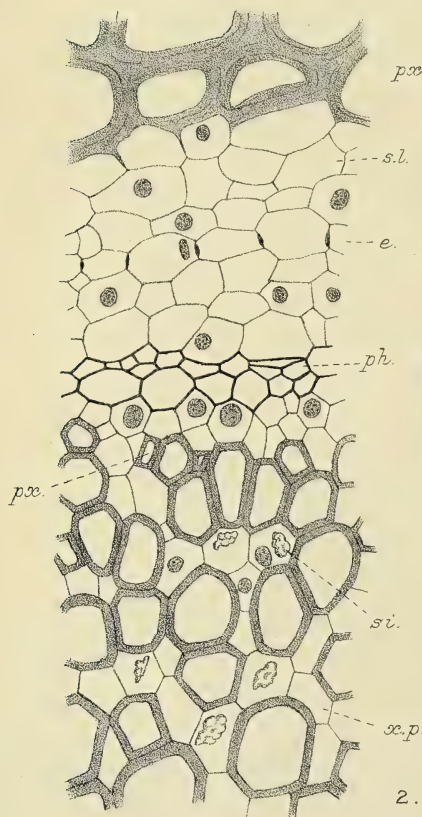
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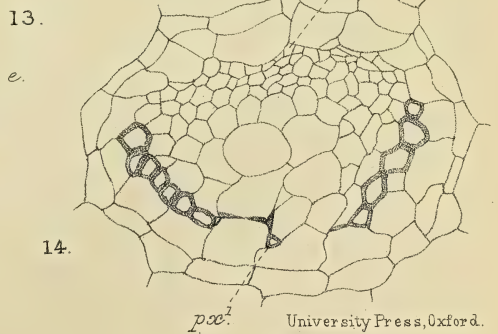
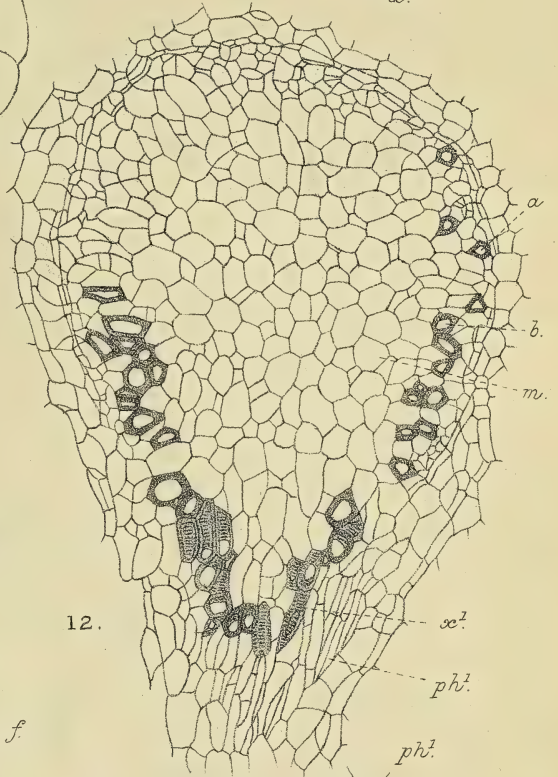
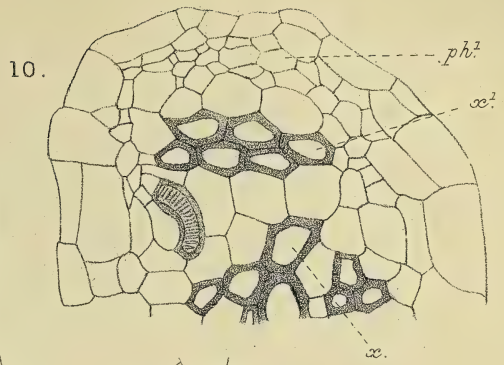
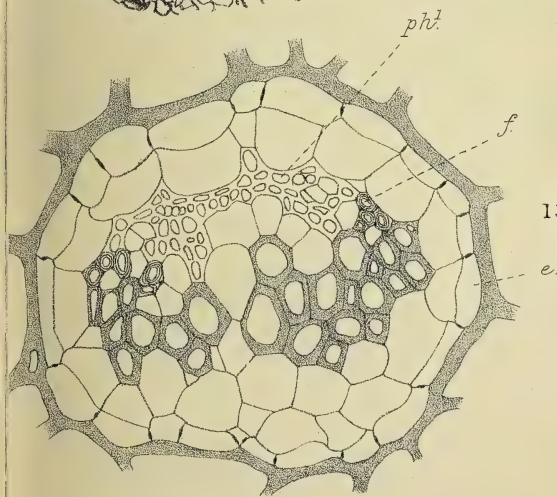
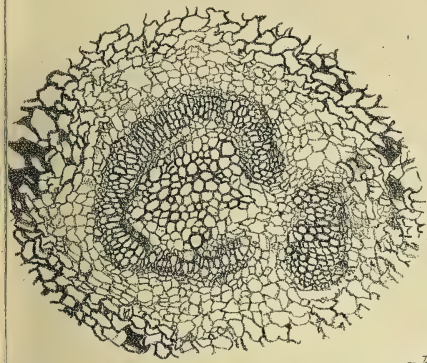
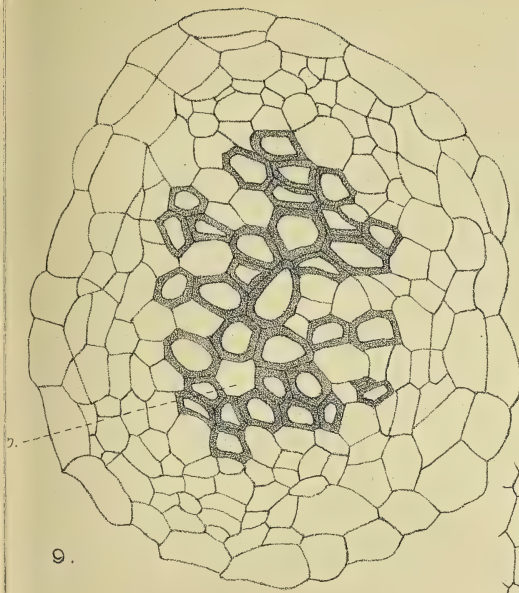


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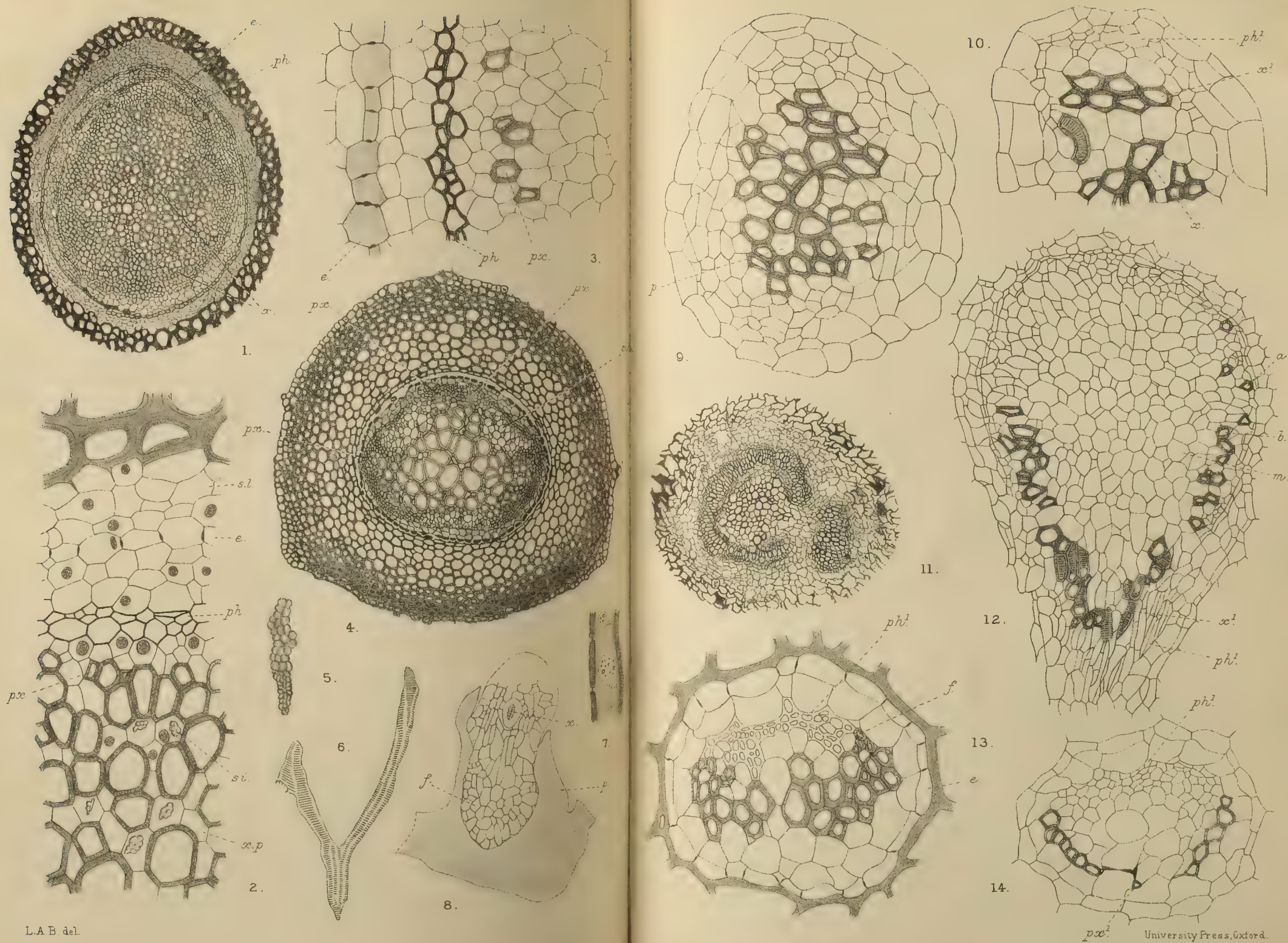


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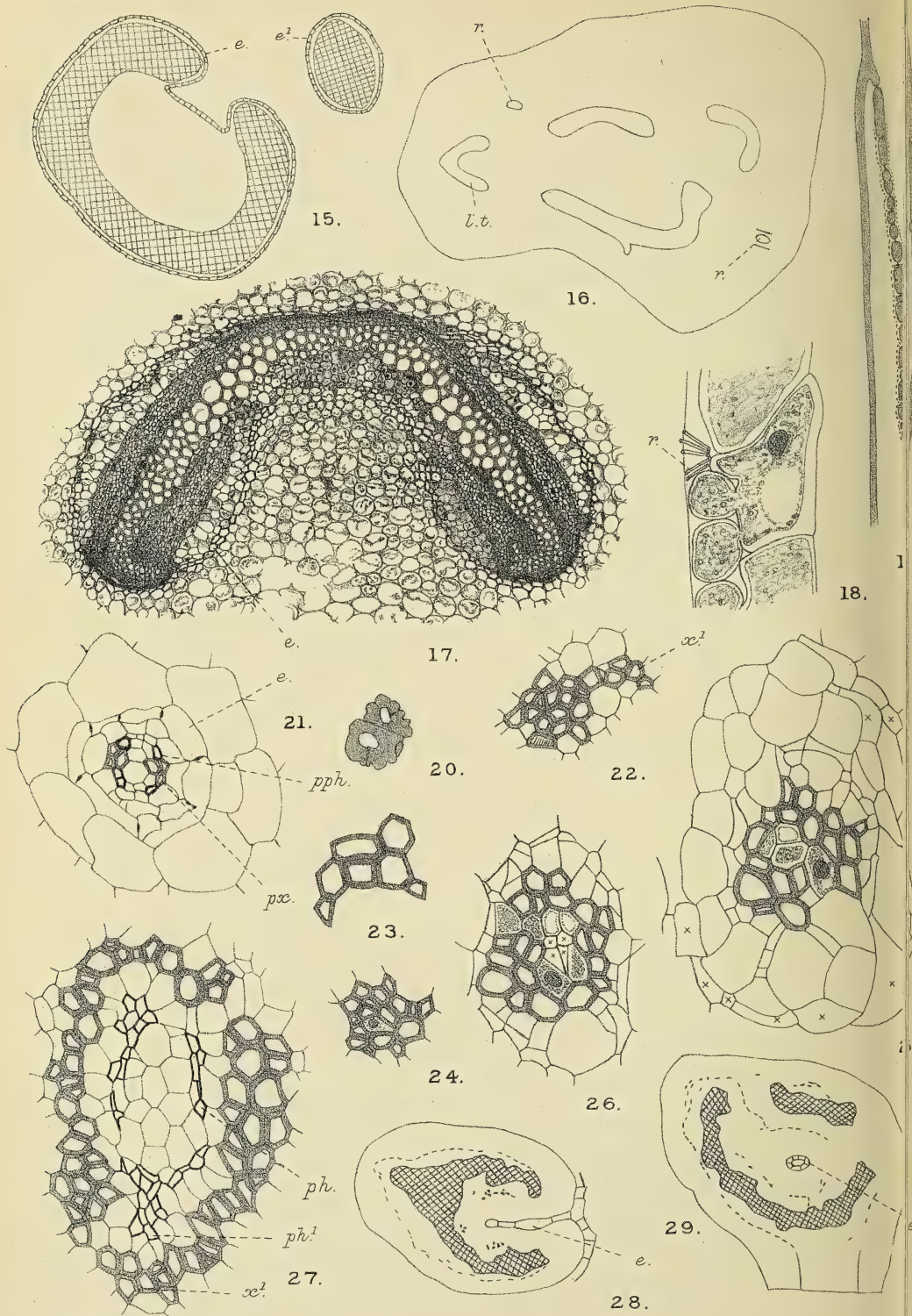
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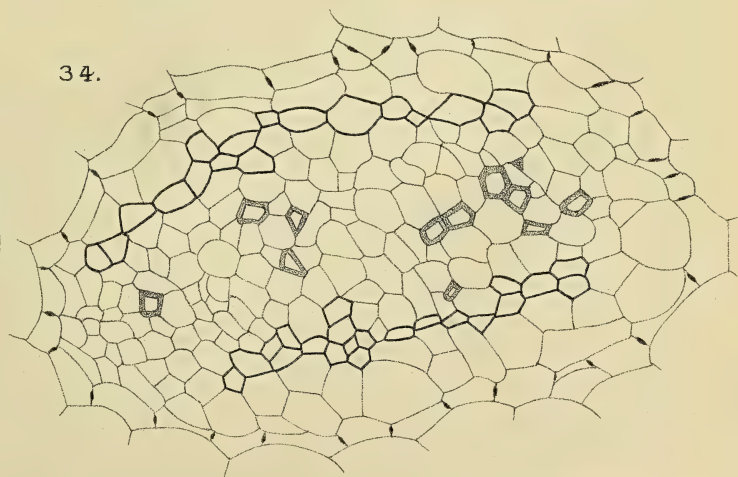
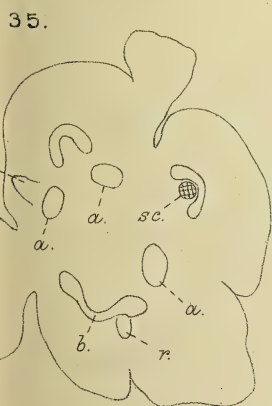
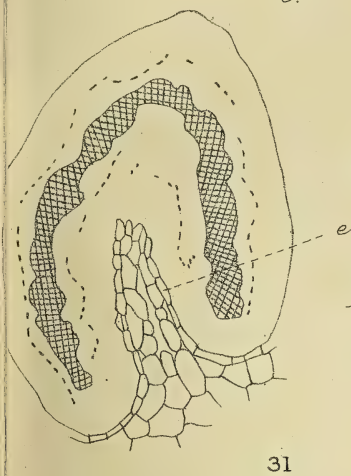
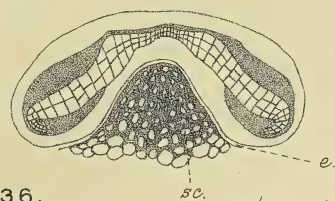
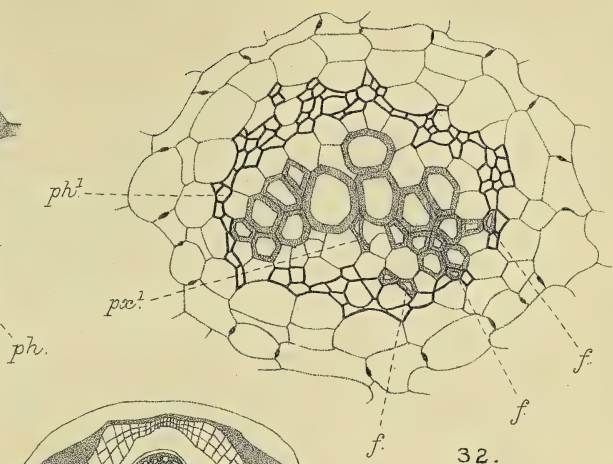
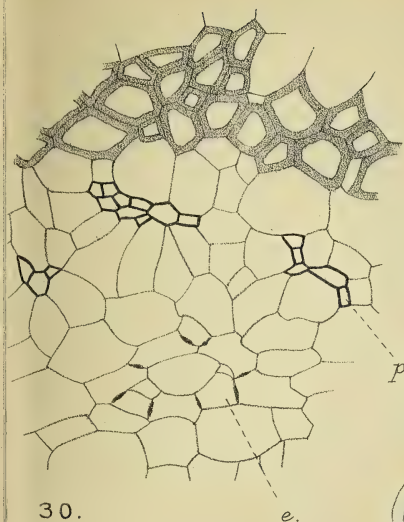


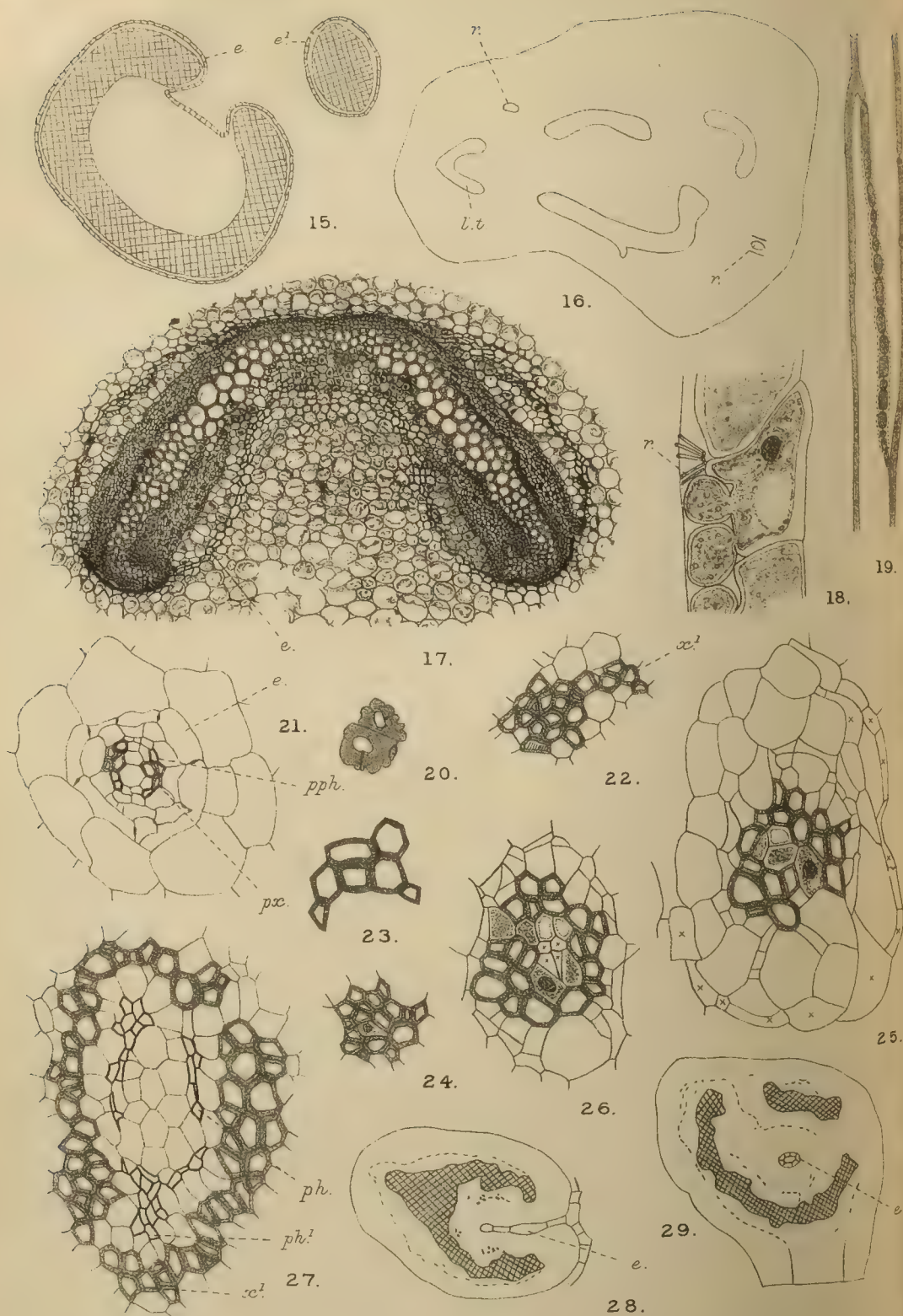


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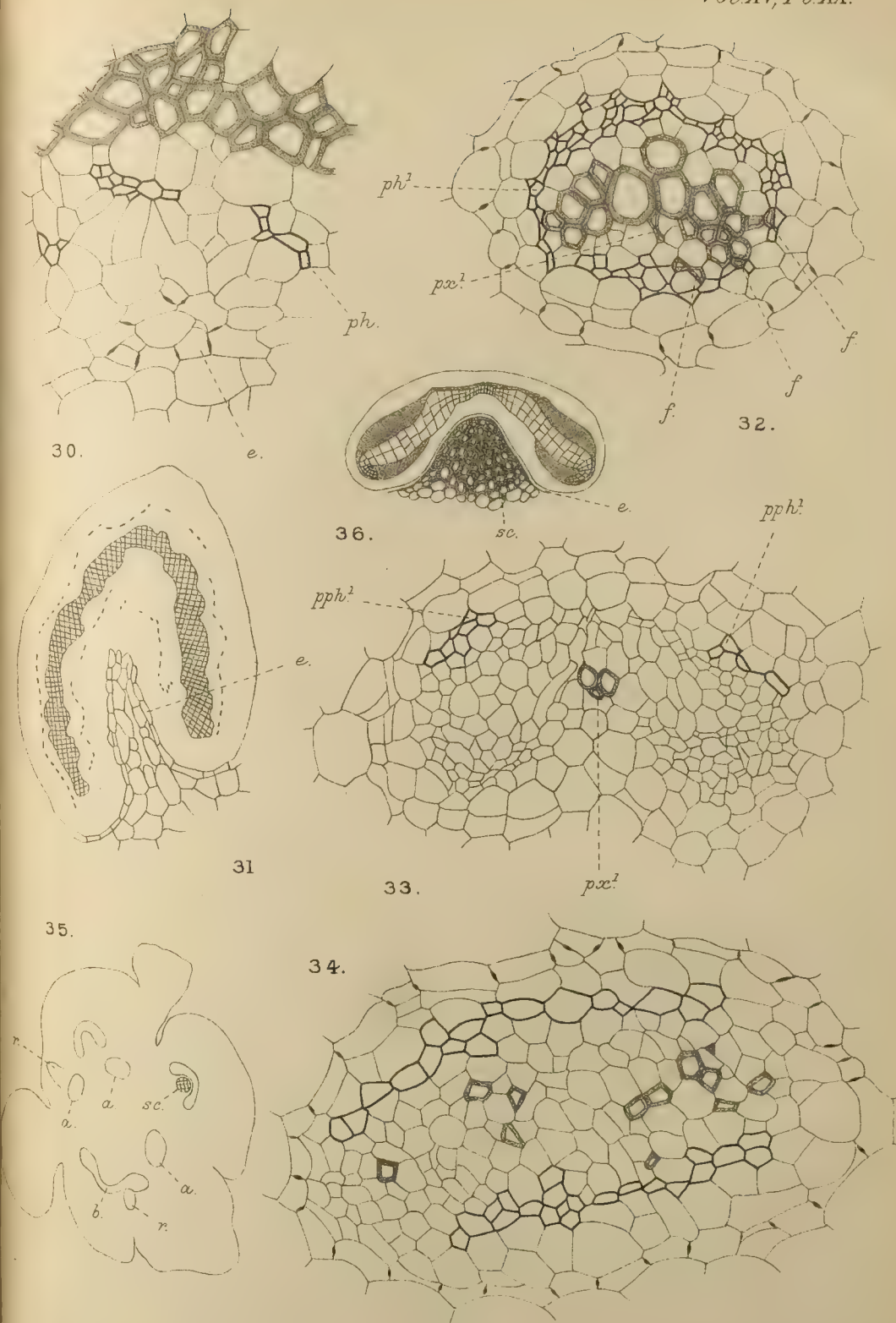


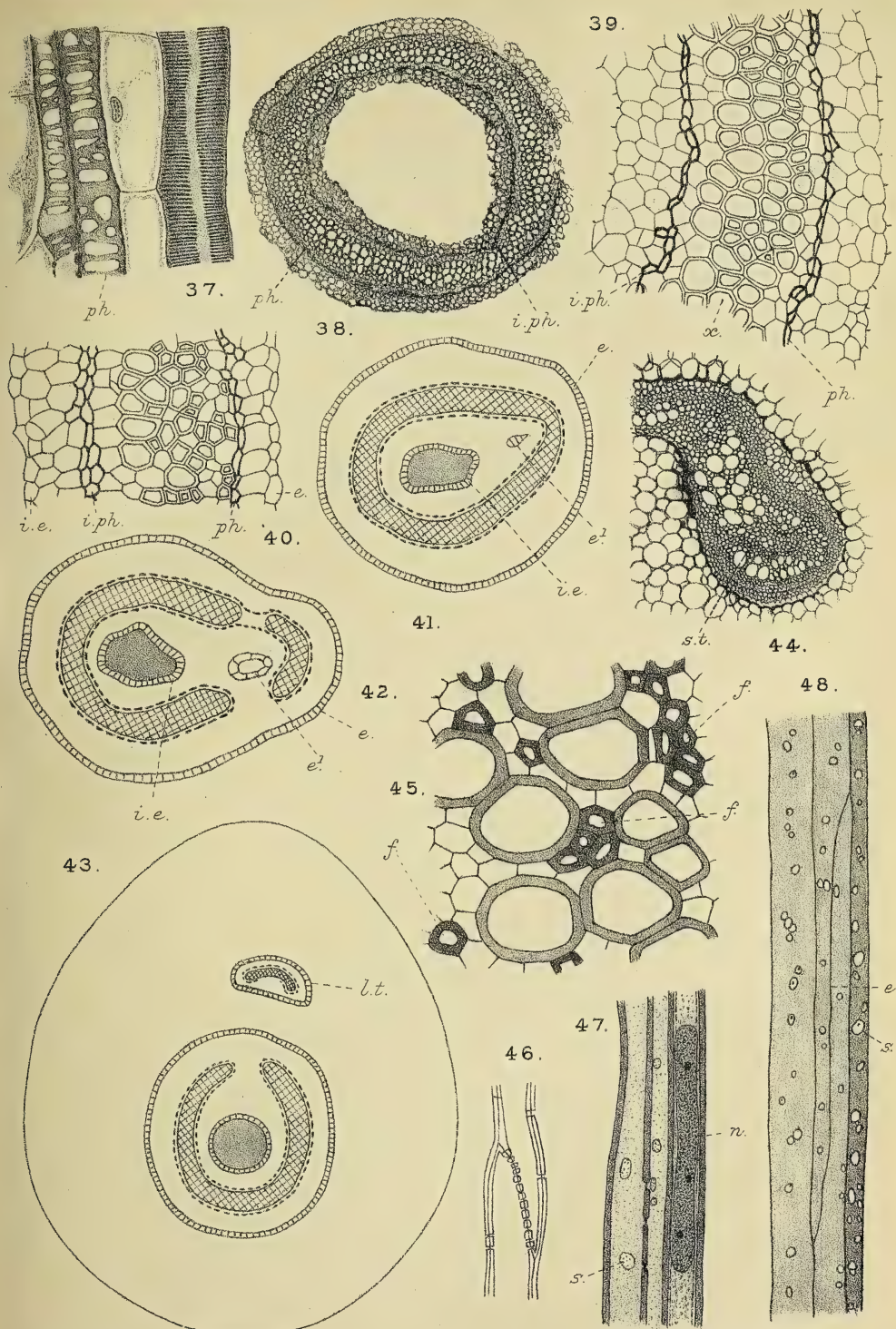
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Morphological Notes.

BY

SIR W. T. THISELTON-DYER, K.C.M.G., C.I.E., F.R.S.,

Director, Royal Botanic Gardens, Kew.

With Plate XXII.

IT frequently happens that interesting specimens come under notice, in a large botanical establishment like Kew, which hardly attract the attention they deserve when relegated to their places in a vast museum collection. Yet, though they rarely afford the bases for any extended research, they deserve detailed notice as they often illustrate important theoretical points, and may be useful to teachers for lecture illustrations.

I propose therefore, from time to time, to put such specimens on record in these notes, contenting myself in most cases with necessary but brief descriptions.

I. PERSISTENCE OF LEAF-TRACES.

The outward extension of leaf-traces is limited by the fall of the leaves to which they belong. It is not, however, apparently generally known that when the leaves are more or less persistent the leaf-traces are continued to them through successive annual zones of wood. This has already been described in detail by Dr. Oskar Markfeldt in 'Flora' for 1885 (pp. 33, 81, and 99):—'Ueber das Verhalten der Blattspurstränge immergrüner Pflanzen beim Dickenwachstum des Stammes oder Zweiges.'

[*Annals of Botany*, Vol. XV. No. LVIII. June, 1901.]

Markfeldt did not, however, have the opportunity of observing the persistence of the leaf-traces in stems of considerable size. It is the object of this note to point out that in such cases they form a conspicuous but unnoticed feature in the wood.

Fig. 3 (Pl. XXII) is a reproduction of a life-size photograph of part of the longitudinal section of the trunk of *Araucaria imbricata*, presented to the museums of the Royal Botanic Gardens in 1890 by the Right Honourable the Earl of Ducie, F.R.S., at whose seat, Tortworth Court in Gloucestershire, it was grown. The trunk from which the specimen was obtained was one foot four inches in diameter at the base. An annual ring has been cut through tangentially and is seen to be perforated by the leaf-traces. The photograph also shows sections of other leaf-traces traversing successive annual rings. In this case the sections are necessarily more oblique. The material does not enable me to ascertain whether the leaf-traces are prolonged indefinitely. But this point will no doubt be cleared up by Mr. Seward, who proposes to make a thorough examination of the trunk of the historic tree formerly living at Kew. This was introduced by Menzies in 1796, but died in 1892.

The same structure is shown no less conspicuously in a specimen of the wood of *Araucaria Cunninghamii* received from the Technological Museum, Sydney, in 1891. It is no doubt a characteristic feature of the wood of every species of the genus. It may be noted that according to De Bary (Comparative Anatomy of the Vegetative Organs of the Phanerogams and Ferns, p. 513), 'the demarcation of the annual rings' in *Araucaria* has been denied by some writers. Fig. 3 shows that in this respect the wood is in no way exceptional.

As far as I am aware this curious structure is peculiar to *Araucaria* amongst Coniferae. I had thought that some indication of it might have been found in *Abies*. But an examination of the extensive series of wood specimens in the Kew museums has failed to detect any evidence of its occurrence.

In *Pinus* a structure may be found which at first sight is similar. Fig. 1 is from a life-size photograph of a piece of 'yellow deal' (*Pinus sylvestris*) taken from an old building at Kew, and without further history. In this case the 'traces' penetrating the annual rings are not mere 'leaf-traces' but are the fibro-vascular cylinders of limited branches.

Fig. 2 shows a transverse section of the same specimen. This has passed through a 'branch-trace,' and shows its termination at the fourth annual ring.

In a specimen of the wood of *Pinus glabra* from N. America, presented to the Kew Museum by Prof. Sargent, the appearance presented in a tangential section near the centre of the trunk closely resembles those that occur in *Araucaria*.

The persistence of leaf-traces affords apparently a decisive character for assigning fossil Coniferous woods in which they occur, at any rate to the *Araucarineae*. Their absence is not however equally conclusive, as I am at present in possession of no evidence that they are continued after the leaves are finally thrown off in very old stems by the disruptive action of circumferential growth. They have, however, been traced to the exterior of the wood in an old stem grown at Kew which shows at least sixty annual rings, and in which all remains of the leaves have entirely disappeared. It is possible therefore that the leaf-traces may continue to extend indefinitely although any foliar function which may belong to them has ceased.

I must express my obligations to Mr. L. A. Boodle, F.L.S., who has taken much kind trouble for me in making the photographs.

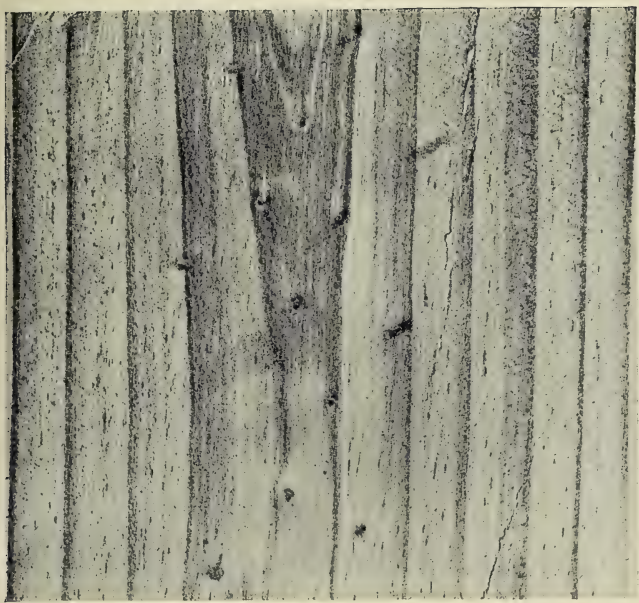


FIG. 1

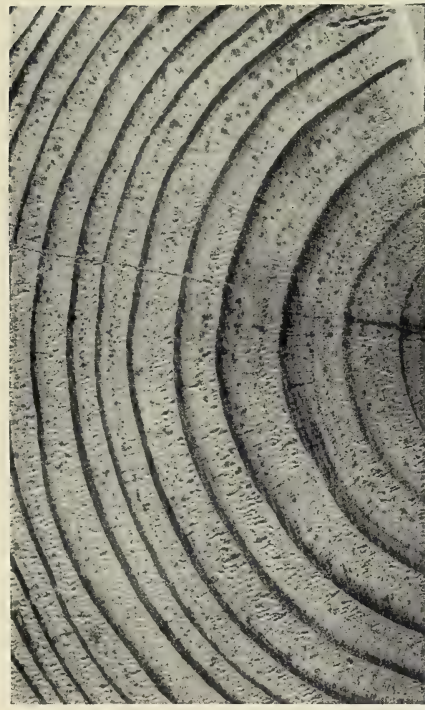


FIG. 2

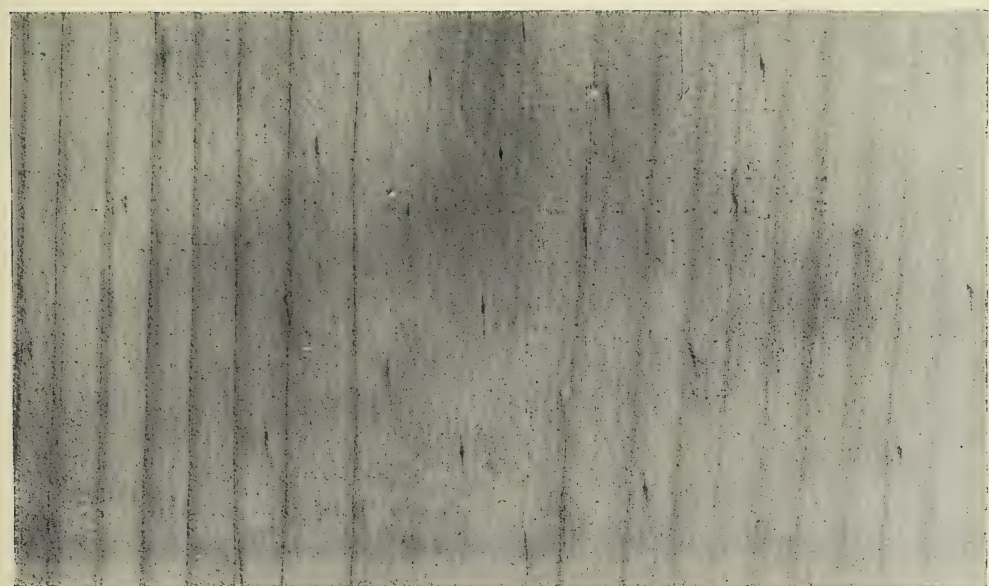


FIG. 3

NOTES.

AN ATTEMPT TO ESTIMATE THE VITALITY OF SEEDS BY AN ELECTRICAL METHOD. By AUGUSTUS D. WALLER, M.D., F.R.S.—The present observations form part of an extensive series of experiments, by which I am engaged in verifying whether or no 'blaze currents' may be utilized as a sign and measure of vitality.

An inquiry of this scope necessitates superficial examination of many varieties of animal and vegetable matter, and the closer study of certain favourable test-cases.

I have selected as such a test-case the 'vitality' of seeds, and have chosen for my purpose beans (*Phaseolus*), which are anatomically convenient and practically easy to obtain of known age.

But before entering upon the results in this particular test-case, I think it advisable to preface those results by a brief indication of the principle involved in all such experiments.

By 'blaze current' (the term which I was led to adopt by the study of retinal effects) I mean to denote the galvanometrical token of an explosive change locally excited in living matter. An unequivocal blaze current electrically excited is in the same direction as the exciting current, i.e. it cannot be a polarization counter-current. (An equivocal blaze current, in the contrary direction to the exciting current, i.e. not at first sight distinguishable from a polarization counter-effect, also exists, but is not taken into consideration in this communication.)

The presence of an unequivocal or homodrome blaze current is in my experience proof positive that the object under examination is

¹ Abridged from the paper in Proc. Roy. Soc., vol. lxxviii, p. 79, 1901.

alive. Absence of the effect is strong presumptive evidence that the object is 'dead,' or rather not-living. It may be in that paradoxical state of immobility which we characterize as latent life, and which we may not characterize as the living state, inasmuch as no sign of life is manifested, nor as dead, inasmuch as the living state can be resumed. An object in this dormant state exhibits no 'blaze current' or other sign of life. And although it has capacity of life, and cannot therefore be classed in the category of 'dead' things, it is not actually living, and must therefore logically be classed in the more extensive category of not-living things.

Limiting ourselves to the unequivocal blaze current as the criterion between the living and not-living states, we may formulate the following practical rule for a summary interrogation of any given object:—

If the after-currents aroused by single induced currents of both directions are in the same direction, the object investigated is alive.

Typical Experiment.—A freshly shelled out and unbruised bean set up laterally between unpolarizable electrodes gives—

1. Blaze currents in the positive direction in response to an induction shock in the positive direction; and in the negative direction in response to an induction shock in the negative direction.

2. The same bean, after removal of a horizontal slice from its under-surface B (giving therefore current of injury of positive direction), gives blaze currents in the negative direction in response to an induction shock in the positive direction (= an equivocal blaze in the polarization direction) and to an induction shock in the negative direction (= an unequivocal blaze in the homodrome direction). If the bean is horizontally sliced at the upper surface A instead of at the lower surface B, the current of injury is negative and the blaze currents positive in response to both directions of excitation.

3. A boiled bean gives no blaze currents in either direction, but only small polarization counter-currents, in the positive direction after a negative current and in the negative direction after a positive current.

The next obvious point to be tested is the effect of anaesthetics upon the response. The results depend upon strength of excitation employed, and duration of anaesthetization. *Cæteris paribus*, the strong effect of a strong stimulus is far more refractory to the action of an anaesthetic than the smaller effect of a weaker stimulus, and in

the former case the suppression is apt to be incomplete, or when complete to be definitive. To obtain temporary suppression it is necessary to choose a sufficient but not too strong exciting current, and to anaesthetize by ether rather than by chloroform.

In a preceding paragraph it has been mentioned that a fresh vigorous seed gives a large blaze current, whereas a stale or moribund seed gives little or no response. The next step was obviously to compare similar seeds submitted to various enfeebling modifications, as well as different crops of similar seeds, the electrical tests being controlled by parallel germination tests.

The first and most readily effected comparison is that between the reactions of fresh seeds and of the same seeds killed by boiling. The result of this comparison is unmistakable and invariable. Fresh seeds giving unequivocal blaze currents with an E.M.F. of 0.01 to 0.10 volt give no blaze currents whatever after they have been boiled, but only polarization counter-current with an E.M.F. of 0.0005 to 0.0020 volt. The seeds upon which I have made this test have been leguminous seeds such as shelled beans and peas boiled in water, and the kernels of stoned fruits such as cherries, plums, and peaches boiled in their protected state.

Table I.—Comparison between beans of the years 1895 to 1899. Forty-eight hours' soakage at room temperature. Average of 10 seeds of each year. Germination test not made.

	1895.	1896.	1897.	1898.	1899.
Weight of 10 seeds—	grammes.				
Before soaking . .	6.2	5.8	6.2	3.3	4.8
After soaking . .	13.9	7.6	12.5	6.4	10.5
Average blaze .	0.0014	0.0036	0.0043	0.0052	0.0170

Table II.—Twelve intact beans of 1895, soaked in water at 24° for twelve hours, then laid on wet flannel in incubator for a further twelve hours at 24°, measured electrically on December 17, and forwarded to Kew for independent test by germination. I have to

thank Sir W. Thiselton-Dyer for the account of their subsequent behaviour.

	<i>Blaze reactions.</i>	<i>Subsequent behaviour at Kew.</i>	
		<i>Date of germination.</i>	<i>Condition.</i>
Bean No. 1 . . .	0.0050	December 28	Weak ¹ .
„ 2 . . .	0.0025	Failed	
„ 3 . . .	0.0175	December 22	Strong.
„ 4 . . .	0.0125	December 27	Moderate.
„ 5 . . .	0	Failed	
„ 6 . . .	0.0100	December 22	Strong.
„ 7 . . .	0	Failed	
„ 8 . . .	0.0100	December 25	Strong.
„ 9 . . .	0	Failed	
„ 10 . . .	0.0050	December 31	Weak ¹ .
„ 11 . . .	0.0100	December 24	Strong.
„ 12 . . .	0.0100	December 24	Strong.

CONCLUSION.

The physiological character of the blaze reaction is proved (1) by the influence of raised temperature ; (2) by its general parallelism with germination tests ; (3) by the influence of lowered temperature ; (4) by the influence of anaesthetics ; (5) by the influence of strong electrical currents ; (6) by the absence of blaze and failure of germination in the case of waterlogged seeds. In every instance a bean giving no blaze gave subsequently no sign of germination.

There has been throughout these first observations a general, but not faultless, correspondence between the blaze reaction and the germinative activity. The correspondence is such as to make good the principal fact that the blaze reaction is a sign of life, and that its magnitude is some measure of what we designate as 'vitality.' The defects of correspondence may have been due to irregularities in the results of the blaze test, or of the germination test, or of both tests. As regards great differences of vitality, both tests are obviously and in every case concordant, both replying by an indubitable 'yes' or 'no'

¹ Those marked weak are not likely to get beyond the cotyledon stage.

to the question whether there is blaze and germination. As regards the lower degrees and the smaller differences of vitality, the chances of disagreement between the two tests are obviously greater. As regards the electrical test, it is more difficult to take the measure upon the entire seed than upon its isolated radicle. As regards the germination test, it is not always easy to ensure identical and optimum conditions.

Fresh and vigorous seeds manifest a large blaze response (0.0500 volt or more), and germinate strongly. Older and less vigorous seeds manifest a smaller blaze (0.0100 volt or less), and a less active germination. Still older seeds, incapable of germination under even the most favourable conditions, manifest still smaller blaze (0.0010 volt or less), and finally none at all, or the small counter-effect due to polarization (0.0005 volt more or less).

THE QUADRIPOLE SPINDLE IN THE SPORE-MOTHER-CELL OF *PELLIA* EPIPHYLLA.—I have read with interest the account of nuclear division in *Pellia* as given by Prof. Davis in the last number of this journal¹, and it is a source of gratification to find that his description of the processes accords so nearly, at any rate as regards matters of fact, with that given by me in 1894 and 1895². But in dealing with the first mitosis in the spore-mother-cell, he has arrived at conclusions respecting the peculiar spindle so characteristic of the earlier stages, which diverge considerably from those which I expressed in the second paper above cited. As the matter is not merely one of detail only, but is of great cytological interest, it seems desirable briefly to examine the evidence adduced by Prof. Davis in support of his own contention.

He apparently regards the quadriple spindle, described by me, as non-existent ('there is no four-poled spindle,' p. 174), and he was not able to trace any structures in the cell of the nature of centrosomes in connexion with the mitosis under consideration. The first of these objections seems to depend chiefly on the mode of interpretation adopted, the latter more nearly touches upon fact.

Now, as regards the failure to identify the centrospheres at this stage, I can only assert that they are certainly to be distinguished in appropriately prepared sections, although they are by no means

¹ Annals of Botany, xv, No. LVII.

² Ibid., viii and ix.

so obvious as those of *Aneura* or, especially, of *Fossombronia*, at the corresponding stage. One reason for this is perhaps to be sought for in the shape of the spore-mother-cell of *Pellia*. The four lobes are much elongated tetrahedrally, and hence only one of the four spindle-rays can possibly, even in the most favourable case, be cut radially, i.e. in such a manner that it can be followed out, in the same section, to the distal end. The rays in the three remaining lobes will obviously present a more or less truncated appearance, such as is actually represented in the Figures 8 and 9 in Plate X. A further obstacle in the way of the recognition of centrospheres lies in the extreme difficulty of properly fixing the cell-contents of the spore-mother-cell of *Pellia* at this stage, and in this respect again it compares unfavourably with the other examples above mentioned. An inspection of some of Prof. Davis' figures, notably 1, 2, and 7, sufficiently proves that he has himself experienced this difficulty.

When discussing the significance of the quadripolar spindles, and their relation with the achromatic figures commonly met with in ordinary cells, I expressly endeavoured to guard myself against the possible misconstruction of having attributed them to any circumstances other than those involved in the unusual form of the cells concerned. Indeed, the chief interest for me in these spindles lay precisely in the possibility of so correlating them. And thus it appeared that the extreme diversity met with in the different genera of the Hepaticae investigated by me was susceptible of an explanation sufficiently general to cover them all (vol. ix, p. 150).

But notwithstanding the evidence furnished by his own figures, Prof. Davis seeks to explain away the existence of the quadripolar spindles by calling them a condition of prophase only, and by implying that the existence of a kinoplasmic web enveloping the nucleus is sufficient to disprove their existence, or at any rate to rob them of all importance. Such *a priori* arguments appear to me to be futile, even were they not sufficiently answered by actual observation of the very definite structures themselves. Quite apart from the existence of centrospheres, it is impossible to disregard the evidence presented by the character of the nucleus and the kinoplasm. Thus a nucleus is shown in Fig. 5, distorted and pulled out tetrahedrally, and the proximal parts of the spindle arms are also represented. The suggestion is put forward that the 'accumulations of kinoplasm most naturally then take up positions of least resistance, and extend

into the lobes of the spore-mother-cells.' But this can hardly be accepted as an adequate explanation, for it implies that the force which causes the nucleus to assume the peculiar shape in question, and which further impels the outward development of the fibrillae, acts from the centre of the nucleus. The evidence, however, here, as in other cases, points not to a centrifugal push, but to a pull acting from without upon the nucleus, at four points corresponding severally to the aggregations of protoplasm situated in the four lobes of the cell.

The further assertion that 'the cones of fibrillae are not spindles' obviously involves a revision of terminology, and is at complete variance with accepted usage. It would be of interest to learn the precise stage at which Prof. Davis considers the fibrillae of the cones, which he figures as running out into the several lobes of the cell, to be transformed into actual spindle-fibres. But the mode of origin of 'the true spindle' is not given, although this is obviously a point of supreme importance in connexion with the views put forward by Prof. Davis.

I may conclude this note by remarking that I see no good reason at present for abandoning the views as to the origin and relations of the spindle structures advanced in the memoirs already cited, and I may be permitted further to express the opinion that, in dealing with complex matters such as those raised by Prof. Davis, the value attaching to general conclusions is very small unless they are founded on a wide and comparative basis.

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LONDON.

LOXSOMA CUNNINGHAMII—A CORRECTION. In vol. xv, No. LVII, *Annals of Botany*, Mr. D. T. Gwynne-Vaughan gives a short review of previous literature upon the anatomy of *Loxsoma*, in which he says (p. 75): 'Finally, Giesenhagen confuses the correct opinion hitherto held regarding the vascular system, by speaking of it as a central collateral or concentric bundle; he also refers incorrectly to the structure of the cortex.'

In view of the fact that the author has given in his paper a most careful description of the anatomy of *Loxsoma*, which adds greatly

to our knowledge of this interesting Fern, it seems to me worth while to correct the error contained in the sentence quoted.

At the time of my investigations of the Hymenophyllaceae in 1889 I excluded *Loxsoma* from my study because proper material was not then at my disposal, and also because I considered this *genus* as not included in the family. I therefore refrained from making any reference to the structure of *Loxsoma*. In the sentence over which the author is troubled this is expressly stated as follows:—

‘Der Stamm der *Hymenophyllaceen*—ich nehme immer *Loxsoma* aus, welches seinem ganzen Aufbaue nach eine besondere Stellung einzunehmen scheint und von den Systematikern neuerdings auch von den *Hymenophyllaceen* getrennt wird—besitzt ein centrales, collateral oder concentrisch gebautes Gefäßbündel. Dasselbe ist umgeben,’ &c.

It is therefore evident that the author has inadvertently failed to grasp my expressed meaning, and has consequently misrepresented my position in this matter, which is precisely that of Prantl, of whom he says—‘no mention is made at all of the anatomy of *Loxsoma*.’

K. GIESENHAGEN.

MUNICH,
April 10, 1901.

In my paper upon the anatomy of this Fern, published in the last number of the Annals, I made a reference to the work of Dr. Giesenhagen on the Hymenophyllaceae, in which, I regret to say, I have, inadvertently, completely misrepresented his statements. At the time I was writing my article I was unfortunately unable to verify this particular reference, so the error was allowed to pass undetected. It is unnecessary to state how much I regret my mistake; it only remains to offer my sincere apologies to Dr. Giesenhagen in the hope that the preceding correction will make clear, and at the same time entirely remove, the erroneous impression I have given of his results.

D. T. GWYNNE-VAUGHAN.

The Development of the Egg and Fertilization in *Pinus Strobus*.

BY

MARGARET C. FERGUSON.



With Plates XXIII, XXIV, and XXV.



DURING the course of the present studies, the development of the archegonium and fertilization, together with the phenomena immediately preceding and following fecundation, were first carefully worked out in *Pinus Strobus*. Later, a far less extended study was made of *Pinus austriaca*, *P. rigida*, *P. resinosa*, and *P. montana*, var. *uncinata*. A complete series of stages in the development of these species was not obtained, and, therefore, the conclusions as set forth in this paper refer, unless otherwise stated, to *Pinus Strobus*, the other species being used only by way of comparison. The methods employed in the preparation of the material for this research were fully described in my earlier paper ('01) and need not be repeated here.

These investigations were begun, at the suggestion of Prof. George F. Atkinson, in the fall of 1897, and the principal results of the work, as herein described, were read before the Botanical Society of America at its Boston meeting in 1898. At this time Blackman's ('98) excellent article, 'Fertilization and Related Phenomena in *Pinus sylvestris*,' had

not appeared. In general, Blackman's observations have been confirmed for *Pinus Strobus*, and other interesting phenomena, not yet recorded as occurring in plants, have been noted.

EARLY DEVELOPMENT OF THE ARCHEGONIUM.

The archegonia in *Pinus*, as is well known, do not make their appearance until the ovules have entered upon the second year of their development. In material fixed during the latter part of May, the cells comprising the upper part of the prothallium, that is, its micropylar end, are seen to be much more regular in shape and arrangement than those which constitute the rest of the endosperm. Cell-division is now taking place in any or all parts of the prothallium, but the regular, more or less rectangular cells in its upper portion are especially active, and they are comparatively rich in protoplasm. Very soon some of the uppermost of these cells cease to divide, but continue to grow, so that they are distinguished from the adjacent cells by their greater size, larger nuclei, and more vacuolate cytoplasm. These are the initial cells of the archegonia (Pl. XXIII, Figs. 1 and 2). At this time the prothallium has, as a rule, become a solid mass of tissue, although it not infrequently happens that there still remains, at its centre, an open space into which the cells of the growing endosperm have not, as yet, extended.

While the primary cell of the archegonium is still quite inconspicuous it divides, giving rise to a small, upper cell, the mother-cell of the neck, and a large, lower cell which forms the venter of the archegonium (Figs. 3 and 4). The small cell immediately divides by an anticlinal wall, and the two cells, thus formed, divide by walls that are perpendicular to the first, the resulting four cells all lying in the same plane. These constitute what may be called the normal neck in *Pinus Strobus* (Figs. 5-9 and 12). Frequently, however, two of these cells divide again, as figured by Strasburger ('69), the six cells being arranged in a single layer (Figs. 10, 25, and 39). Occasionally all four cells divide by anticlinal walls, the neck then consisting of eight cells, all of which lie in the same

plane (Pl. XXIII, Figs. 3, 11, and Pl. XXIV, Fig. 40); in rare instances, the four cells divide by periclinal walls, when the eight cells which compose the neck of the archegonium are disposed in two tiers of four cells each (Fig. 20). This last seems to be the normal condition in *Pinus austriaca* and *P. rigida*, and is that figured by Blackman for *Pinus sylvestris*.

At first the growth of the central cell is not followed by a corresponding increase in the amount of protoplasm; and hence its cytoplasm early presents a very vacuolate appearance. There may be one large, irregular, central vacuole; or delicate strands of cytoplasm may extend out from the peripheral layer of the protoplasm, forming vacuoles of various sizes. But, as the central cell continues to enlarge, its cytoplasm begins to develop more rapidly, many strands extending out into and across the vacuoles; thus the size of the vacuoles is decreased while their number is greatly increased. The central vacuole, if present, may persist for a considerable time, or it may be replaced at once by smaller vacuoles (Figs. 4-7). Gradually the cytoplasm becomes more dense; and the vacuoles, receding from the periphery of the cell, especially from its base and sides, disappear last from its upper portion (Figs. 8 and 9). When the ventral canal-cell is cut off the vacuoles have nearly or quite been replaced by a finely granular cytoplasmic reticulum, in which a greater or less number of larger, more deeply staining granules are embedded. These granules are frequently surrounded by a clear court into which the protoplasmic network has not extended. The number of the so-called proteid vacuoles is usually small at this time (Fig. 10).

The nucleus of the central cell attains full size very soon after its formation. It has a delicate, more or less interrupted, reticulum, and is characterized by a large, vacuolate nucleolus which invariably occupies a central position; one or two smaller nucleoli may also be present. This nucleus always remains close beneath the neck cells, and is more or less concave on the side towards these cells (Figs. 4-9, 13, 14, and 16). Although, as Blackman has pointed out, the vacuolate

nature of the cytoplasm renders this nucleus very liable to displacement during the early stages in the development of the archegonium, yet, in well-fixed material, it is always found in its normal position. Hirase ('95) states that certain granules, which appear in the cytoplasm just beneath the nucleus of the central cell in *Ginkgo*, have been derived from the nucleus or from its nucleolus; and Ikeno ('98) describes the nucleus of this cell in *Cycas* as giving out a granular substance during its growth period. No comparable phenomenon has been observed in connexion with the nucleus of this cell in the species of pines which I have studied; but, as above stated, the nucleus quickly reaches its mature size, and remains apparently unchanged until the inception of its division.

Very early in the history of the archegonium, the cells immediately surrounding it become differentiated from the adjacent endosperm-cells by their more regular form, the greater density of their cytoplasm, and the increase in the size of their nuclei. Thus a distinct sheath, consisting as a rule of a single layer of cells, is formed about the venter of the archegonium. No special attempt has been made to count the number of chromosomes in the nuclei of the various parts of the sporophyte and gametophyte; but whenever a nucleus was observed in which the chromosomes were particularly clear and distinct their number was always noted. In such cases twelve chromosomes have invariably been found in the nuclei of the sheath-cells. Chamberlain ('99) has found the same number in the corresponding cells of *Pinus Laricio*.

The early development of the archegonium, as just described, agrees in the main with that given by Strasburger in 1878.

As the archegonium grows, the prothallium also continues to increase in size, several layers of cells being formed above the archegonium, except over its neck-cells. Here no prothallial tissue is laid down, and hence there arises an opening in the endosperm leading from the neck-cells to the nucellar cap (Figs. 9-12). In the last stages of development, the sides

of this tubular cavity often become very closely crowded together so that the passage is obscured.

The number of archegonia in a single ovule varies in *Pinus Strobis*, *P. rigida*, and *P. resinosa* from one to five, the most usual number being three. In *Pinus austriaca* and *P. montana*, var. *uncinata*, the number is larger, averaging about five; as many as nine have been observed in a given prothallium in *Pinus montana*, var. *uncinata*. The form of the mature egg depends largely upon the number and arrangement of the archegonia. When there are not more than two or three, as is frequently the case in *Pinus Strobis*, they may become almost spherical in outline.

DIVISION OF THE CENTRAL CELL.

As the central cell prepares for division, the cytoplasm between its nucleus and the neck-cells is apparently resolved into fine granules, and there is a more or less pronounced condensation of the protoplasm about the lower side of the nucleus. At the same time the nucleolus disappears wholly or in part, the nuclear reticulum becomes more open and broken, and the chromatin collects or condenses at various places on the network (Fig. 14). Soon a clear court, similar to that described by Hof ('98), Fulmer ('98), Nemec ('98 and '99), Strasburger (1900) and others, makes its appearance along the lower half of the nucleus; inasmuch as this nucleus is pressed close against the neck-cells such a court does not arise along its upper side (Figs. 15 and 16). Delicate, granular threads cross this court and press against the nuclear membrane; at the same time the upper and lower surfaces of the nucleus become irregularly indented (Fig. 17). As the chromatin condenses to form the spirem, an achromatic network, as already described for the corresponding stage in the division of the generative nucleus in *Pinus*, becomes apparent in the nuclear cavity (Figs. 14-17). When the spirem is fully established it presents a beautiful moniliform appearance and the longitudinal splitting of the band becomes apparent; at this time the threads which earlier arose in the cytoplasm

seem to have been again resolved into granules (Fig. 18). Whether any of them enter the nuclear cavity and contribute to the formation of the achromatic spindle has not been definitely ascertained. The spindle, when formed, lies wholly within the nucleus. During the early metaphase of the division the nuclear membrane can still be distinguished, and clearly consists of a web of threads (Figs. 20, 21).

When the spindle arises it is 'multipolar in an axial plane,' and thus corresponds, with slight variation, to the mitotic figure described by Duggar ('00) in the microspore of *Symplocarpus foetidus*, and by Wiegand ('99) in the microspore of *Potamogeton foliosus*. In *Pinus*, however, the upper extremities of the threads do not at first unite into groups, but remain practically free, and are closely pressed against the neck-cells (Fig. 20). The several poles, formed at the inner or lower extremity of the karyokinetic figure, soon draw together, forming a single, very sharply defined pole; or the fully developed spindle may remain more or less truncate at its lower end. Blackman describes this spindle as bluntly truncate at both extremities. I have frequently observed such a spindle during a late anaphase of the division, but this is only one of the various aspects which may be presented during metakinesis and later stages in this mitosis. The upper extremities of the achromatic spindle-fibres may never draw together at all; they may unite to form two or more poles, or they may give rise to one pole which may be blunt or very slender (Figs. 22-26). But whatever form may be assumed by this spindle during the later stages in its development, there is always formed, at an early period, a diarch spindle (Strasburger, '00) which is multipolar at one extremity and monopolar, or nearly so at the other (Figs. 20 and 21). A like condition also exists at an early stage in the division of the generative nucleus in the pines; and it is suggested that such a figure may be characteristic, at least in the higher plants, of those indirect divisions which result in the formation of nuclei of different sizes.

The chromosomes, when oriented at the nuclear plate, are

invariably in the form of U's or V's. Blackman states that they are straight rods, but he does not so figure them. The cell-plate, during the early stages in its formation, lies midway between the developing nuclei, but when the daughter-nuclei are fully formed the nucleus of the oosphere is, as a rule, farther removed from the cell-plate than is the nucleus of the ventral canal-cell. It is very early evident that the cell-plate consists of two layers. As Chamberlain ('99) has shown, the lower portion of the spindle at this time is ordinarily convex, while the part within the ventral canal-cell is concave (Figs. 30 and 32).

I was able, in several preparations similar to that illustrated in Fig. 23, to count the number of chromosomes, and twelve or thirteen were found in both groups instead of eight as counted by Dixon ('94).

THE VENTRAL CANAL-CELL.

As a rule the nucleus of the ventral canal-cell never presents a normal appearance, but shows signs of disintegration very early in its history. It is doubtful, in some cases, if a nuclear membrane is ever formed, and there are probably instances in which fusion of the chromosomes never takes place at all (Figs. 25 and 27); although Blackman, judging from such an appearance as that shown in Fig. 29, holds the latter condition to be impossible. The nuclear membrane, when present, very soon breaks down, and the chromatic substance becomes scattered throughout the cell (Figs. 30-32). This cell, immediately preceding and at the time of fertilization, ordinarily forms a deeply staining mass which lies just beneath the neck-cells and above, but in contact with, the egg (Figs. 12, 32, 40, and 42). The nucleus of the ventral canal-cell in *Pinus austriaca* has twice been observed to approximate in size that of the egg nucleus (Fig. 29), but in a study of several thousand archegonia of *Pinus Strobus* no instance has been found in which these two nuclei were similar in form; the nearest approach to a normal nucleus

that has been observed in the ventral canal-cell of this species is that shown in Fig. 28. Occasionally this cell is somewhat enlarged and is furnished with a rather scanty amount of cytoplasm in which distinct chromosomes, or chromatic figures of various forms, are embedded (Figs. 83 and 84, Pl. XXV). There seems to be a definite relation between the structure of the ventral canal-cell and the character of the upper part of the mitotic figure formed in the division of the central cell. This is clearly demonstrated on a comparison of Figs. 22-29.

The separation of the canal-cell from the cytoplasm of the oosphere, as Strasburger ('72) and Blackman ('98) have described in *Pinus*, is, I believe, due to a shrinkage of the egg-protoplasm caused by imperfect fixation; and it is possible that a similar appearance in *Cycas*, Ikeno ('98), has a like origin.

MATURATION OF THE EGG.

The egg-nucleus is no sooner formed than it begins to increase in size, becoming greatly enlarged even before the disappearance of the spindle-fibres (Figs. 27-31). As the nucleus moves toward the centre of the oosphere, threads of more or less delicacy extend, in a radial manner, from its wall into the surrounding cytoplasm. These fibres are not equally well defined in all preparations, but, whatever the degree of their prominence, they are invariably more strongly differentiated about the upper side of the nucleus, and may extend from the nucleus to the top of the egg (Figs. 32, 34, and 35).

As already stated few, if any, vacuoles persist within the venter of the archegonium at the time of the division of the central cell. Following their disappearance there arise numerous spherical bodies, the so-called proteid vacuoles. Co-ordinate with the downward movement of the egg-nucleus these bodies assume a position about the periphery of the oosphere, more especially at its base (the organic apex of Strasburger), and sides (Figs. 11, 12, and 41). Under a low

power, the cytoplasm of the mature egg appears dense and finely granular; the 'proteid vacuoles' do not seem to differ materially from the protoplasm in which they are embedded; and many deeply staining granules are scattered throughout the cell. With greater magnification, however, a very beautiful, granular reticulum becomes apparent; there is no suggestion of the alveolar structure described by Butschli ('94). At times this reticulum is everywhere crossed by short fibres, which have no definite arrangement, and are, apparently, not confined to any fixed period in the history of this cell (Fig. 30). The spheres in the outer and basal portions of the cytoplasm are resolved into very complex structures which, although they simulate the appearance of nuclei, could never be mistaken for such bodies by one familiar with cell-structures (Figs. 32 and 34).

Immediately preceding fertilization a cavity appears in the egg-cytoplasm, just beneath, or in the near vicinity of, the neck-cells (Figs. 12 and 38). In some cases this opening may not arise until the instant of fertilization. This cavity, which was thought by the earlier writers to represent the lower portion of the pollen-tube within the oosphere, has been explained by Blackman ('98) as due to the sudden inrush of the contents of the pollen-tube, and by Arnoldi ('00) in *Cephalotaxus*, as caused by the downward movement of the conjugation-nucleus. Shaw ('98) suggests that the concavity in the upper part of the egg of *Onoclea*, just prior to fertilization, may correspond to the receptive spot; and there is every evidence that in *Pinus Strobis* this opening in the cytoplasm represents the last act of the egg in its preparation for the reception of the sperm-nucleus. If it were formed by the movement of nuclei or other bodies through the protoplasm, we should expect the cytoplasm to draw together again, as during the downward movement of the egg-nucleus; but, in reality, this opening persists throughout the entire later history of the archegonium (Pl. XXV, Figs. 77 and 78). The regular, clear outline of this cavity, together with the fact of its presence in the unfertilized as well as the fertilized

egg, warrants one in considering it a definite character of the mature oosphere.

No cytoplasmic radiations, similar to those described by Belajeff ('91) in *Taxus baccata* and by Dixon ('94) in *Pinus sylvestris*, have been observed in connexion with the fully developed egg-nucleus in any of the species of Pines which I have studied.

During the growth and downward movement of the egg-nucleus, it never presents a definite network, such as is observed in the nucleus of the ordinary resting-cell; but it is characterized at a very early stage by an open, interrupted reticulum, on which are arranged irregular granules of various sizes. This meshwork may be extremely delicate; it may assume a heavy appearance; or it may become very much interrupted and broken, many detached portions lying loose within the nuclear cavity (Figs. 27-36). Nucleoli have rarely been observed in this nucleus in *Pinus Strobus* during the first stages of its development (Figs. 27, 28, 30, and 31); but in *P. austriaca* they occasionally arise very early (Fig. 29). When the nucleus has attained considerable size, small nucleolus-like bodies, containing a single central vacuole, appear in connexion with the nuclear net; and at the same time a slightly larger nucleolus is observed in the lower part of the nucleus, usually in connexion with its membrane (Fig. 32). As the nucleus continues to grow, this nucleolus also increases in size, gradually becoming large and very vacuolate (Figs. 34-36).

When the egg-nucleus reaches maturity, it has attained huge dimensions, and its outline, depending on the form of the egg, is spherical or elliptical. The nucleolus, if demonstrable, is always found in the lower part of the nucleus; and there are usually several smaller bodies, designated in this paper as secondary nucleoli, scattered throughout the nucleus (Fig. 36). These secondary nucleoli are invariably found in connexion with the reticulum, but, as Montgomery ('98) believed, they are probably caught in, not vitally united to it. They may be present in great abundance, or they may be entirely

absent from the nucleus. The reticulum, on which the chromatic substance is disposed, presents numerous aspects, as already indicated in the description of this nucleus during its period of growth. Under very high magnification, it does not show, in normal conditions, a true granular structure; but it may present a most delicate, interrupted, granular network; or it may consist of large, irregular, diffusely staining masses which are united into an imperfect reticulum (Figs. 37*a* and 37*g*). In the latter instance the chromatic granules are either too minute to be distinguished, or they have been dissolved in the linin ground-work. The linin, always very abundant in this nucleus, may form heavy hyaline cords, on which the chromatin is collected at irregular intervals (Figs. 37*e* and 37*f*); but it more often consists of less conspicuous strands (Figs. 37*b*-37*d*). Great as the variations are in the structure of this nucleus, its chromatin has always been found, in the species of Pines studied by the writer, to exist either in the form of irregular granules of varying sizes or apparently dissolved in the linin. Such a resolving of the chromatin into nucleoli as that described by Chamberlain ('99) in *Pinus Laricio* and illustrated in his Figs. 14 and 15 has not been observed.

Whether the various appearances presented by the egg-nucleus represent normal phases in its life-history, or whether one is normal and the others artefacts resulting from the action of fixing agents, is, of course, a mere matter of conjecture. But inasmuch as these different aspects are characteristic of this nucleus during its period of growth, also after it has to all appearances reached maturity, and again at the time of its conjugation with the sperm-nucleus, it seems reasonable to conclude that all are normal and correspond to certain physiological activities which take place within the nucleus.

Strasburger ('84) described the nucleus of the oosphere in the Abietineae as being densely filled with a granular substance, which entirely obscured or masked the chromatin. This substance he called metaplasm, and virtually considered

the nucleus a vacuole filled with a nuclear sap capable of taking up or elaborating this material. Ikeno ('98) found a similar substance in the sexual nuclei in *Cycas*, and Arnoldi ('00) in *Cephalotaxus*. Blackman ('98) devoted several paragraphs to a discussion of metaplasma, as it manifested itself in the egg-nucleus of *Pinus sylvestris*. He found that it was present in the young nucleus in the form of granules, but that it later united with the chromatin to form the nuclear reticulum. Chamberlain ('99) does not recognize the presence of this substance in the egg-nucleus in *Pinus Laricio*; and there is no evidence of its existence in the sexual nuclei of the species of Pines studied by myself.

According to Wilson ('99), 'protoplasmic substances represent the active, metaplastic structures the passive elements' of the cell. During the development of the egg-nucleus in the species of Pines which have formed the basis of these studies, there is never any deposit within the normal nucleus of a granular substance; but the linin, as already stated, becomes very abundant. Just what proportion of it is active in cell-division we are unable to say. Without doubt a large part of the linin merges into the cytoplasmic network during the first segmentation of the oosphere-nucleus, but even so it cannot be classified with the passive elements of the cell.

Blackman ('98) wrote: 'The stage in which the nucleus is found in a position between the apex and the centre of the egg is rarely met with'; and Chamberlain ('99) stated 'that in over three hundred preparations, less than a dozen' show early stages in the development of the egg-nucleus. During the course of these investigations upon the Pines, more than twenty-five hundred preparations, representing several thousand archegonia, have been studied, and no developmental stage has been more frequently met with than that by which the nucleus assumes its central position in the egg. Such an appearance as that illustrated by Chamberlain in his Figs. 18 and 19 has often been observed in both the young and the mature egg-nucleus, in the conjugating nuclei, and also in the various nuclei of the proembryo. They have been wholly disregarded

in the present discussion of the maturation of the egg, for, in our material, these figures, and also Blackman's Fig. 11, would be interpreted as representing disintegration stages. Every step has been repeatedly traced from the ordinary nuclear reticulum to nuclei which can scarcely be distinguished from the surrounding cytoplasm, and then to archegonia which appear perfectly normal, except that no nuclei can be demonstrated within them. It is a well-known fact that the number of seeds derived from a Pine cone is very small in comparison with the number of ovules formed in a given cone. An examination of fresh material shows that development may cease at any point between the early stages in the formation of the ovule and the last steps in the ripening of the seed. This cessation of growth does not at once become apparent, and so cannot be avoided, in its earliest stages, when one is putting up material for cytological work. Under such conditions, it is inevitable that, with a limited amount of material, the abnormal will be interpreted for the normal.

The entire development of the archegonium in *Pinus Strobos* is passed through in about two weeks, probably not more than five days elapsing between the cutting off of the ventral canal-cell and fertilization. In *Pinus montana*, var. *uncinata*, these processes are apparently much more closely united in point of time, as the pollen-tube, in some cases, has reached the endosperm before the division of the central cell is completed (Fig. 33).

CONJUGATION OF THE SEXUAL NUCLEI.

When the time for fertilization arrives, the apex of the pollen-tube is ruptured, and almost all its contents pass into the cytoplasm of the egg. The sperm-nuclei, still surrounded by a common mass of protoplasm; the vegetative nucleus; the stalk-cell; a part of the cytoplasm from the pollen-tube; and some of the starch-grains from the male gametophyte can all be distinctly recognized in the upper part of the oosphere (Figs. 39-42). Dixon ('94) noted the passage into

the oosphere of the four nuclei of the pollen-tube, but he could not distinguish between these after their entrance into the egg. Blackman confirmed Dixon's observations as to the passage of these nuclei into the oosphere, and believed that the cytoplasm of the 'sperm-cells' passed into the egg along with the sperm-nuclei, but he was unable to demonstrate the fact. There can be no doubt that the cytoplasm of the sperm-cell enters the egg in *Pinus Strobus* (Fig. 39). This cytoplasm very soon fuses with that of the egg, and the larger sperm-nucleus moves towards the nucleus of the oosphere; the other elements from the pollen-tube remain for some time in the upper part of the ovum. There is no evidence that the sperm-nucleus increases in size after entering the oosphere; neither is there an increase in stainable substance, but, on the contrary, the nucleus loses its dense structure; and occasionally a nucleolus becomes apparent within it. (Compare the sperm-nuclei in Figs. 39 and 40 with those in Figs. 42-50.)

There is every indication that the movement within the egg of the sperm-nucleus which becomes active in fertilization is both rapid and direct. It almost invariably traverses the shortest distance between its point of entrance into the egg and the egg-nucleus. The relative position which the conjugating nuclei may occupy with reference to the major axis of the oosphere varies considerably, but always bears a definite relation to the position of the neck-cells. When these cells are directly above the centre of the oosphere, the sperm-nucleus comes into contact with the upper part of the egg-nucleus (Figs. 41, 44, 45, 48, and 50); but if the neck be excentrically placed, the sperm-nucleus will be found against one side of the oosphere-nucleus (Figs. 43, 46, and 47). It has not been observed beneath the egg-nucleus as figured by Coulter ('97) in *Pinus Laricio*. Neither is there a bulging of the egg-nucleus towards the sperm-nucleus, nor do the sexual nuclei ever approximate in size as shown in this same figure of Coulter's; but a somewhat similar figure has been observed in *Pinus Strobus* after the first division of the

'segmentation-nucleus.' The sperm-nucleus is usually described as being more dense than the egg-nucleus at the time of their conjugation, and this is sometimes found to be the case in *Pinus Strobus*; as a rule, however, the conjugation-nuclei differ in size only, as observed by Arnoldi ('00) in *Cephalotaxus*.

Just before the sexual nuclei come into contact, the side of the egg-nucleus adjacent to the sperm-nucleus becomes slightly concave (Fig. 43). This concavity is doubtless formed under the influence of the approaching sperm-nucleus, and suggests the crater-like depression developed at an earlier period in the egg-nucleus of *Cycas* (Ikeno, '98). As noted by Blackman, the sperm-nucleus does not penetrate the membrane of the egg-nucleus; it lies in a pocket-like indentation formed in the side of the oosphere-nucleus, both nuclei then occupying the space originally filled by the egg-nucleus. The sperm-nucleus, when in contact with the nucleus of the egg, ordinarily assumes the form of a biconvex lens, but it may vary much in outline, presenting in some cases the figure of a crescent, and in others that of an ellipse. Occasionally it forms a deep, tongue-like depression in the nucleus of the oosphere (Figs. 41-50).

THE FIRST DIVISION FOLLOWING FECUNDATION.

While the sexual nuclei lie in intimate contact, but still, to all appearances, perfectly distinct, certain changes in their structure indicate that each is in the early prophase of division. The chromatin condenses or collects in irregular granules about the periphery of the sperm-nucleus, while that of the egg-nucleus is deposited just beneath the sperm-nucleus. The remainder of each nucleus is filled with a granular, achromatic reticulum of great beauty, reminding one of delicate frost-work (Fig. 51). This condition suggests an early stage of fertilization in the sea-urchin as described by Wilson ('95). Wilson thinks that the sudden increase in linin may be only apparent, resulting from the

'rapid condensation and localization of the chromatic substance'; but he is inclined to believe that 'a considerable portion of the chromatin breaks down at this time into linin.' It would appear that the prominence of the achromatic reticulum in the conjugating nuclei of *Pinus* results from both these processes. For, while there is always a large quantity of linin in the egg-nucleus and a comparatively small amount of chromatin, the size of the chromatic spirem, when formed, seems disproportionate to the entire bulk of the chromatin earlier existing in the nucleus.

The chromatin continues to separate out from these nuclei until a spirem, studded with irregular granules, lies just within the wall of the sperm-nucleus, and a similar one arises directly below in the egg-nucleus. Frequently the cytoplasm caught between the two nuclei collects into spherical masses; between these spheres of cytoplasm the membranes of the nuclei come into close contact (Fig. 52). Very soon the spirem of each nucleus becomes coiled and regularly moniliform; and the chromatic band of the sperm-nucleus takes up a position along that side of its nucleus which is nearest to the spirem formed in the egg-nucleus. At this time delicate, minutely granular threads, some of which pass from nucleus to nucleus, appear in the regions of the two chromatic spirems. The rest of the achromatic contents of these nuclei is largely transformed into long, comparatively heavy threads, which are furnished with innumerable granules. The two nuclei are still perfectly distinct; the nucleolus of the egg-nucleus may persist at this stage; and the nuclear membranes are yet present, although they are very irregular in outline, and have given way at several points (Fig. 54). The nucleolus is not always present at this time, but nucleolus-like masses, which from their position are evidently derived from the egg-nucleus, may be present as late as the telophase of the division. Delicate, granular fibres continue to arise in the regions of the two spirems; the coarser, achromatic threads of the nuclei become finer in structure, and extend in all directions towards the forming spindle; and the nuclear membranes fade entirely

out, not only along the line of contact of the two nuclei, but from their entire outer surfaces as well (Fig. 55). Blackman found that, while the chromatic portions of these nuclei remained distinct in *Pinus sylvestris*, the nuclei fused at an early stage in the prophase of the division. There is, apparently, no such fusion of the sexual nuclei in *Pinus Strobos*; but the entire membrane of each nucleus disappears during an early prophase of the mitosis, and the contents of the nuclei lie free in the cytoplasm of the egg.

The spindle-fibres continue to increase in number, becoming even more delicate in structure, and losing their granular appearance. The long, now quite delicate, but still granular, achromatic threads of the nuclei are very numerous, and many extend into the areas occupied by the chromatic spirems. They probably feed the growing spindle, some of them, doubtless, being directly transformed into spindle-fibres. The chromatic bands have now become perfectly homogeneous. Before their segmentation, the very irregular, multipolar polyarch spindle has become a multipolar diarch (Strasburger, '00) spindle; and the achromatic substance not used in spindle-formation has been gradually resolved, from the periphery of the nucleus inwards, into a granular, or finely reticulated structure, which later merges into the general cytoplasm of the egg (Figs. 56-58). When the spindle has become a true multipolar diarch, it frequently consists of two nearly equal parts, which seem to belong respectively to the male and the female nucleus (Plate XXV, Figs. 58 and 59). This appearance, however, may be only accidental, as the great irregularity which characterizes this spindle in the first stages of its formation renders such an origin of the two halves of the nearly completed spindle very problematic.

Two chromatic groups are distinctly recognized at the time of the segmentation of the spirems, and can still be clearly made out during the early development of the chromosomes (Figs. 59 and 60). When the chromosomes are oriented at the nuclear plate, the maternal and paternal elements can no longer be distinguished (Fig. 61). One

beautiful preparation was obtained at this stage in which a single section through the nuclear plate showed twenty-four entire chromosomes, and no chromosomes were found in the other sections of the series (Fig. 62). As twelve chromosomes had previously been counted in the egg-nucleus, there can be little doubt that the same number is brought into the egg by the sperm-nucleus.

The smallness of the mitotic figure in the first division following fecundation compared with the size of the egg-nucleus has been commented upon by all students of the *Abietineae*. This spindle may occupy various positions in the space originally filled by the egg-nucleus; but, as is clearly demonstrated by a study of its development, it invariably lies partly within the sperm- and partly within the egg-nucleus, its major axis being always parallel with the outer free surface of the sperm-nucleus. While, then, the division-figure bears a certain definite, fixed relation to the conjugating nuclei, it will be readily seen that its position may vary, depending upon the shape of the sperm-nucleus and its line of contact with the egg-nucleus, as, also, upon the plane at which the section is cut with regard to the sexual nuclei. For instance, when the sperm-nucleus is elliptical in outline, and lies in a deep depression in the egg-nucleus, as illustrated in Fig. 48, the spindle will appear to occupy the centre of the egg-nucleus. Cases like the above and many others were first satisfactorily interpreted after a careful study of something like two hundred preparations showing fertilization-stages.

During matakinesis the mitotic figure may present every variation between the extremely broad, multipolar diarch, shown in Fig. 63, and the narrow, almost bipolar spindle, illustrated in Fig. 64. It is at this time that the longitudinal splitting of the chromosomes first becomes apparent. Each chromatic element divides at the point where the spindle-fibres are attached, forming a small diamond-shaped opening. While this opening is still inconspicuous, the two halves of each chromosome become distinct throughout the entire length of the segment. Such a condition was several times

observed in the division of the 'segmentation nucleus,' but was not sketched because of lack of space. A similar stage in the division of one of the four nuclei of the proembryo is shown in Fig. 80 *b*.

In general the chromosomes at the nuclear plate are in the form of U's and V's; in rare instances they are long and somewhat coiled, and the spindle-fibres are not attached to their centres (Figs. 61-64). They pass to the poles as narrow U's (Fig. 66). Sometimes the arms of the U are pressed so closely together that the chromosomes look like longitudinally split rods. In a late anaphase of the division, the chromatic elements present a crinkled appearance, and the poles of the spindle terminate in granular areas from which threads extend into the surrounding cytoplasm. These fibres may be quite inconspicuous or they may be very prominent, frequently forming fantastic figures (Figs. 67 and 68).

A portion of the achromatic constituents of the sexual nuclei may persist in the region of the mitotic figure until the formation of the daughter-nuclei, but, as a rule, all traces of the original nuclei have disappeared at this time. Blackman finds no suggestion of a cell-wall in connexion with the first division which takes place within the oosphere. But here, again, great variation has been found in *Pinus Strobilus*. The spindle either becomes constricted at the centre with little or no sign of thickening along its median line, or it may be very broad, in which case prominent thickenings occur, only to disappear at a later stage, in the line of the cell-plate (Figs. 66 and 69). As the half-chromosomes unite to form the daughter-nuclei, the poles of the spindle often become very slender, and seem to press against the forming nuclei, rendering them concave along their inner surfaces; and delicate fibres now extend from all sides of the division-figure into the cytoplasm (Fig. 69). As already indicated, there is no evidence that any portion of this spindle is derived from the cytoplasm, and it is probable that a large part, if not all, of its fibres are formed by a rearrangement of a portion of the achromatic, nuclear reticula. During the dissolution of the

mitotic figure, some of the substance of the spindle-threads probably passes into the daughter-nuclei, but the greater part of the fibres merge into the cytoplasmic reticulum and become indistinguishable from it. We have here another evidence that cytoplasmic and nuclear elements are but different expressions of the fundamental or ground substance of the cell. When the daughter-nuclei are formed, they present very beautiful, moniliform reticula, which later undergo changes very similar to those described for the growing egg-nucleus.

As recorded by Wilson ('96 and '00), Van Beneden ('83 and '87) made the very interesting discovery, confirmed by Herla ('93), that the chromosomes are formed separately in the sexual nuclei of *Ascaris megalocephala*. The differentiation of the chromatic segments takes place after the entrance of the sperm-nucleus into the egg, but before the two nuclei have come into contact. Thus the exact equivalence of the chromatic substance in the paternal and maternal nuclei was demonstrated. In the following year, Strasburger ('88) suggested that in the coming together of the nuclear threads lay the important point in fertilization. A separating-out of the chromatic elements similar to that described by Van Beneden, has since been found to occur during fertilization in many animals, but has not yet been demonstrated as of frequent occurrence in plants. In 1891, Guignard described the formation of two distinct chromatic spirems in the conjugation nucleus of *Lilium Martagon*, but he did not figure them, and his statement seems to have been overlooked by most later writers. Strasburger was able, in 1897, to distinguish the maternal and paternal portions of the fertilized nucleus in *Fucus* up to the time when the spindle was fully formed. But the results of more recent writers¹ seem to indicate that

¹ Arnoldi ('00) in *Cephalotaxus*, Caldwell ('99) in *Lemna*, Campbell ('99) in *Sparganium*, Farmer and Williams ('98) in *Fucus*, Guignard ('99) in *Lilium*, Harper ('00) in *Pyronema*, Ikeno ('98) in *Cycas*, Jäger ('99) in *Taxus*, Land ('00) in *Erigeron* and *Silphium*, Lotsy ('99) in *Gnetum*, Merrell ('00) in *Silphium*, Mottier ('98) in *Lilium*, Mottier ('00) in *Dictyota*, Nawaschin ('99) in *Lilium*, Nawaschin ('00) in *Helianthus*, *Delphinium*, and *Rudbeckia*, Osterhout ('00) in *Batrachospermum*, Shaw ('98) in *Onoclea*, Thom ('99) in *Adiantum* and *Aspidium*,

fertilization in plants consists in the fusion of two nuclei to form a resting nucleus.

Students of *Pinus*, however, have attained quite different results, and find, in this genus, phenomena very similar to those occurring during fertilization in some animals. Blackman concludes that in *Pinus sylvestris* 'no resting fertilized nucleus is ever formed,' and that 'the half-chromosomes, derived from the male and female nuclei respectively, fuse together at the poles of the first segmentation-spindle'; and Chamberlain found that two chromatic spirems were formed in *Pinus Laricio*, but, as so many stages were lacking in his material, he hesitated to draw definite conclusions. As a result of the present studies, it is found that, in *Pinus Strobus*, the chromatic portions of the sexual nuclei remain distinct until the daughter-nuclei are formed; and there is never any true fusion of the conjugating nuclei; i.e. the two nuclei do not form one individual enclosed by a definite membrane.

No centrosome or centrosome-like body has been observed in connexion with the sexual nuclei, either before or during this division. Although the centrosome as an organ has failed to be demonstrated, yet a detailed study of this mitosis makes the conclusion inevitable that the *force* initiating and controlling the division is supplied by the sperm- and not by the egg-nucleus—this force manifesting itself only in the presence of the egg-cytoplasm.

THE DIVISION OF THE TWO SEGMENTATION-NUCLEI.

The two daughter-nuclei remain in the upper part of the egg and pass through the same stages in their development as those described in the maturation of the egg-nucleus, except that, as a rule, no nucleolus becomes apparent within them. These nuclei have been observed to approximate in

Thomas ('00) in *Callha*, Wager ('00) in *Peronospora*, and Wuiczki ('99) (according to Arnoldi and to reviews in the Bot. Zeit. and in the Journ. Roy. Micr. Soc.) in *Larix*.

size to the mature egg-nucleus; but they usually cease to grow and begin to divide while they are still much smaller than the fully developed nucleus of the oosphere. The steps in the division of these two nuclei are almost exactly like those of the first division. The nuclear reticulum is resolved into a beautiful, open and interrupted, granular, achromatic network, which is crossed by several coarsely granular, deeply staining threads. These threads, which represent the chromatic portion of the nucleus, have at first no definite arrangement; but they soon unite to form two distinct coiled or angled spirems which draw together at one side of the nucleus (Figs. 71 and 72). It is an interesting fact that these spirems are always found on the adjacent sides of the two nuclei. This position suggests that there is a certain attraction, comparable to that existing between the sexual nuclei, active between these nuclei; or the relation of the inner sides of these nuclei with the poles of the spindle, in the early stages of their formation, may have some influence upon the position which these spirems assume in the dividing nuclei.

When the two spirems, which are still roughly beaded with the chromatic substance, come to lie side by side along the inner wall of the nucleus, the nuclear wall resolves itself into a web of fibres. These threads pass into the surrounding cytoplasm and soon wholly disappear; at the same time, achromatic fibres arise in the regions of the spirems (Figs. 72 and 73). These threads quickly draw together, forming a sharply bipolar spindle on which the two now perfectly homogeneous, chromatic bands lie. The spindle does not become bipolar in some instances until after the segmentation of the spirems (Figs. 72-74). We have preparations representing a very complete series in this division, but, as it is exactly similar, especially in its later stages, to the first division, it is thought best not to multiply sketches by repeating like figures.

There can be little doubt that the two spirems formed in each of these nuclei represent the separated-out paternal and maternal chromatic substance, although, to all appearances,

the chromosomes were completely fused in the reticula of the daughter-nuclei. One is reminded by these phenomena, of Strasburger's ('92) remark, when he states that he accepts the view of a complete fusion of the segments into a network in the daughter-nuclei, and then asks if he must, therefore, conclude that the chromosomes in the following divisions do not correspond in material.

Rückert ('95) found that the chromatic portions of the conjugating nuclei in *Cyclops* not only remained distinct during the first division, but that two groups of chromosomes, representing respectively the maternal and paternal chromatic elements, could still be recognized after several divisions had taken place. In this case, however, the two groups do not fuse in the daughter-nuclei but a double nucleus is formed in the resting stage. In the same year Zoja ('95) observed that in *Ascaris* the maternal and paternal chromosomes remain entirely distinct during several successive divisions of the segmentation-nucleus. We have, then, in this second division, a further point in which fertilization-phenomena in *Pinus* correspond to those which occur within the ova of some animals. I have, as yet, made no attempt to obtain a complete series of stages in the development subsequent to the formation of the first four nuclei of the proembryo. But, from a comparison of Fig. 79 *b* with 71, and 80 *b* with 64, one is led to expect that the third division following fertilization will correspond in all points with the second. It would be interesting to determine if two chromatic groups are characteristic of all the divisions which normally occur within the oosphere of *Pinus*, and I hope to be able, shortly, to investigate this question more thoroughly.

THE FOUR SEGMENTATION-NUCLEI.

As a rule these nuclei retain their position in the upper half of the egg until their growth is completed (Fig. 76). Here, again, as in the development of the two segmentation-nuclei, the steps described for the maturation of the egg-

nucleus are repeated, except that a nucleolus does not generally become apparent within these nuclei. When they have attained full size they pass to the base of the oosphere. During their descent many fibres arise in the cytoplasm surrounding the nuclei. Some of these threads run parallel with the walls of the nuclei, while others extend out from the nuclei in a radial manner. These fibres become more prominent as the nuclei approach the base of the oosphere, and, as in the case of the egg-nucleus, they are most strongly developed along the upper sides of the nuclei (Figs. 77, 77 *b*, 78, and 78 *b*). When these nuclei have nearly reached the bottom of the egg, the nutritive spheres have almost disappeared from the cytoplasm, those which still persist being much reduced in contents (Fig. 78). After the four nuclei have arranged themselves at the 'organic apex' of the oosphere, in a plane perpendicular to the major axis of the archegonium, a marked change occurs in the cytoplasm of their immediate vicinity. It becomes dense, coarse, more or less granular, and has a great affinity for stains (Figs. 79 and 79 *b*). Blackman describes the formation of cell-walls between these nuclei, but, in the five species of pines which I have studied, cell-walls do not arise until after eight nuclei have been formed.

The early prophases, as also the meta- and ana-phases in the mitosis of the four segmentation-nuclei, correspond in every respect with the same stages in the second division following fertilization; and it is probable that the chromosomes are derived from two distinct spirems as in the first and second divisions occurring within the egg; but, as already indicated, the steps in the origin and development of the chromosomes have not been carefully traced in this division. These nuclei divide simultaneously. Chamberlain states that 'in the division of the four nuclei the spindle is extremely broad and multipolar.' I have occasionally observed such a figure during this mitosis, but here, again, great variation exists. Every transitional form may be presented during metakinesis between a multipolar diarch spindle, which fills

the entire breadth of the nucleus, and a slender bipolar spindle, such as is shown in Fig. 80 *b*. As the halves of each chromosome separate at the point where the spindle fibres are attached, the longitudinal splitting of the segments becomes evident throughout the entire length of the chromosomes (Fig. 80 *b*).

During mitosis, the deeply staining substance surrounding these nuclei condenses into large irregular masses. When the eight nuclei are formed, this deeply staining material collects about them and extends in irregular strands into the cytoplasm. Each nucleus is now surrounded by its own cytoplasm, though no cell-walls have yet been laid down (Figs. 79 *b*-80 *b*). The deeply staining, cytoplasmic substance appears to be repelled from all sides of these nuclei and is deposited in lines which indicate the position of the future cell-walls; the cell-membranes appear to arise by a direct transformation of this substance. The process seems to be very similar to that described by Farmer and Williams ('98) in *Fucus*. Mottier ('00) inclines to the view that the cell-plate is deposited in the form of a homogeneous fluid, the kinoplasm, even though its presence cannot be demonstrated, being the active agent in its deposition. The substance which is cast out, or passes out, from the region of the eight nuclei in the formation of cell-walls at the base of the oosphere in *Pinus*, has the appearance at times of a homogeneous, deeply staining fluid, in which numerous irregular granules are embedded; but there is never any evidence of its being purely fluid in nature.

The origin and disappearance of the achromatic spindle in the several divisions, which have been followed within the oosphere of *Pinus*, indicate that, as in the division of the generative cell of the pines, so here, the spindle-fibres are not the expression of a special kinoplasmic substance.

THE SO-CALLED PROTEID VACUOLES.

The true nature of the proteid vacuoles is a subject which attracted my attention very early in the course of these investi-

gations. There can be no doubt that there is an intimate relation between the sheath-cells of the pines and the substance of the egg, such as is believed to exist between the follicle-cells and the egg in animals. But the exact nature of this connexion, in *Pinus*, is not easily determined. I have rarely examined a preparation showing archegonia without studying the relation of the sheath-cells to the oosphere; and yet no entirely satisfactory evidence, because not demonstrable beyond question, of the origin and nature of the so-called proteid vacuoles has been found.

Hirase ('95) observed that the granules in the egg of *Ginkgo* were of nucleolar origin, being derived both from the nucleus of the central cell and from the nuclei of the sheath-cells. Arnoldi ('00) found that substantially the same thing was true in *Cephalotaxus*. He was not able to detect the passage of the nucleoli from the sheath-cells into the egg, but, since these granules were present on both sides of the membrane of the egg-cell, he accepted the fact of their transference. I have frequently seen a nucleolus partly without and partly within the nucleus of a sheath-cell; but in no instance could one be sure that such a condition was not the result of mechanical displacement.

Ikeno ('98) found direct evidence that the nutritive spheres in *Cycas* are of nuclear origin. But no such phenomenon as he observed in *Cycas* occurs in *Pinus*. Platner ('86) described the passage of the follicle-cells into the ovum in *Helix*, and a few other such instances have been recorded in animals. Arnoldi ('00) has recently noted a most remarkable migration of whole nuclei from the sheath-cells into the egg in several species of pines. He has observed, in a single series, as many as one hundred and fifty nuclei passing into the ovum. From the fact that Arnoldi writes *Strobis* in a parenthesis after *Pinus Peuce*, I infer that he employs the terms as synonyms; but I find no authority for such a usage, and cannot accept his conclusions as holding good for *Pinus Strobis*. It does not seem possible that, in a careful examination of several thousand archegonia, so obvious

a phenomenon as that described by Arnoldi could have escaped detection; and I must, therefore, conclude that it does not take place in the species of pines which I have studied.

Some interesting observations have been made, however, regarding the nature of the nucleolus of the egg-nucleus. As already indicated, this nucleolus does not arise in *Pinus Strobos* until the egg-nucleus has attained considerable size. It appears in the lower part of the nucleus as a minute, solid, spherical body; during growth a small, central vacuole appears, then other vacuoles, until, at maturity, it is completely filled with vacuoles of various sizes (Figs. 32-36). A limiting membrane is not always apparent in this nucleolus (Fig. 80, Pl. XXV); but, in some instances, there seems to be very strong evidence of such a membrane (Figs. 36, Pl. XXIV, and 90, Pl. XXIII). In Fig. 36 the nucleolar wall has been broken at one place, and a vacuole, lying near the point of rupture, has been indented along its outer surface, thus becoming crescent-shaped. Montgomery ('98) sounded a word of warning against interpreting the peripheral stratum of the ground-substance of the nucleolus as a wall-layer; and there is a possibility that, in the figures above referred to, what appears like a limiting membrane is only the outer, unmodified portion of the nucleolus.

The attitude of this nucleolus towards dyes varies much at different periods in its history. It may or may not take the safranin stain characteristic of Flemming's triple combination; it may stain intensely with gentian-violet or iron-hematoxylin (Figs. 36, Pl. XXIV, and 88, Pl. XXIII); it may show a weak reaction to these stains (Fig. 90, Pl. XXIII), or it may be absolutely unaffected by them, remaining as a hyaline or greenish-yellow structure (Fig. 89, Pl. XXIII). When the nucleolus resists the action of dyes, its nucleus is usually totally free of the secondary nucleoli, which have been described in connexion with the maturation of the egg-nucleus, and the cytoplasm of the egg is studded, to an unusual degree, with large, deeply staining granules. But

the nucleus containing a nucleolus which stains with avidity, generally contains, also, innumerable secondary nucleoli; at the same time there are comparatively few deeply staining granules in the cytoplasm of the egg.

The position of the secondary nucleoli with reference to the primary nucleolus is frequently such as to indicate that the former originate in the latter (Figs. 54, Pl. XXIV, and 88, Pl. XXIII). The only observations which would militate against such an origin are the few cases found in which the secondary nuclei seem to appear earlier than the primary nucleolus (Fig. 29). It may be that, in these cases, the primary nucleolus has not yet become differentiated in structure from the secondary nucleoli, as would evidently be true in a stage slightly younger than that shown in Fig. 32; or it may be true that the primary nucleolus is present, but fails, at this time, to stain.

The nuclei of the cells surrounding the young archegonia contain from three to five nucleoli, and one or more nucleolus-like structures may be present in the cytoplasm of these cells. Each nucleolus is surrounded by a clear court which, as Zimmermann ('96) has pointed out, is evidently not an artefact. These nucleoli may be spherical, elliptical, irregular, or long and almost dumbbell-like in outline. The ordinary cells of the prothallium do not now show nucleoli. If such bodies be present, they are small and obscured by the nuclear reticulum. At about the time of the cutting off of the ventral canal-cell, many small, nucleolus-like masses appear in the nuclei of the sheath-cells—twenty or more occurring in a single nucleus. When the egg has reached maturity, and during the later stages of its history, no nucleolus, or but one or two nucleoli, can be demonstrated in the nuclei of the sheath-cells. These nucleoli are no longer surrounded by a hyaline court, but are embedded in the chromatic network.

The nucleoli of the sheath-cells present the same attitude towards stains as does the nucleolus of the egg-nucleus. But while the nucleoli of the sheath-cells frequently stain but feebly, they rarely fail entirely to stain.

During an earlier study of the sporogeny of *Pinus Strobus*, similar colour-reactions were observed in connexion with the nucleoli. The occurrence of unstained nucleoli in the same nucleus in which others were deeply coloured was common, especially at about the time of synapsis.

Various views are held regarding the nature of the nucleolus. We cannot here enter into a discussion of the voluminous literature dealing with the origin, function, and destiny of these structures; but a few of the many views which have been advanced may be noted.

Strasburger ('95, '97, and '00) expresses his conviction that nucleolar substance contributes to the formation of spindle-fibres. A similar view is held by Fairchild ('97), Harper ('97), Debski ('97), and other students of the Bonn Laboratory. Strasburger further holds that the nucleoli make active the spindle-forming substance in the cytoplasm, or that they enhance the activity of the kinoplasm.

Flemming ('82), Humphrey ('95), Zimmermann ('93), Sargent ('96 and '97), Duggar ('99), Mottier ('00), and many others believe that the nucleoli represent reserve supplies of chromatin. Dixon ('99) finds in them a vehicle of inheritance. Hirase ('98) thinks that they give rise to the attractive spheres; and according to Karsten ('93), Lavdowsky ('94), and Wilcox ('95) they are centrosomes.

Jordan ('93) states that 'their function is almost certainly one of nutrition either concerned in the storage or elaboration of nutritive material'; Lukjanow ('88) and Macallum ('91) consider the nucleoli to be excretory organs which are intimately related to the nutritive spheres of the egg, these spheres arising through a process of deposition from the nucleolus; and Häcker ('93) observes that the nucleolus is a contractile vacuole which absorbs proteid substances. The absorbed materials undergo a chemical change within the nucleolus, and are then periodically discharged.

Fleming ('82), Zacharias ('85), and Zimmermann ('93) ascribe to the nucleolus the dignity of a nuclear organ; and Montgomery ('98) makes the following suggestion: 'That though

the nucleolus consists of substances which stand in some relation to the nutritive processes of the nucleus, and so, at the time of its formation, may be a functionless, inert mass of matter, yet it may at later periods in the history of the resting nucleus, acquire some active function, and thus gradually come to acquire the value of a nuclear organ.'

The nucleolus of the egg-nucleus, as also the nucleoli of the sheath-cells in *Pinus Strobus*, appear to represent active portions of the cell rather than inert masses of matter. Certain aspects presented by these nucleoli are surely suggestive of plastids. The uncoloured framework of the egg-nucleus reminds one very strongly of a chlorophyll-body from which the pigment has been extracted. Yet we would not, in the present state of our knowledge, denominate them plastids. I believe, however, although the phenomena are not of such a nature as to admit of definite demonstration, that the nucleolus of the egg-nucleus, as also the nucleoli of the sheath-cells, is actively engaged in the formation of a substance which in the egg-nucleus assumes the shape of secondary nucleoli. These nucleoli become diffused throughout the nucleus, from which they pass, probably in solution, into the egg-cytoplasm. Here they are again differentiated, and by a gradual development, give rise to the 'proteid vacuoles' or nutritive spheres of the oosphere. It may be that the greater size of the egg-nucleus, in comparison with that of the sperm-nucleus, is correlated with the physiological rôle, as above suggested, which it plays in the cell.

THE FATE WITHIN THE EGG OF THE SMALLER SPERM-NUCLEUS, THE VEGETATIVE NUCLEUS, AND THE STALK-CELL.

When these nuclei first enter the oosphere there is no question as to their identity, to one who has become familiar with them before their exit from the pollen-tube (Figs. 40-42). Remnants of these cells have been found in the upper part of the egg as late as the formation of the eight-celled stage of the proembryo. The stalk-cell remains for some

time unchanged, and finally disintegrates. In so far as I have been able to determine, it assumes a more or less granular appearance, and at last blends with the cytoplasm of the egg. The vegetative nucleus undergoes various changes. Occasionally it seems to contract, becoming gradually smaller until it is no longer demonstrable; it may change little, if at all, in size, but its reticulum often becomes more prominent than when within the pollen-tube; rarely it enlarges rapidly after its entrance into the egg and develops a beautiful reticulum (Fig. 39). The sperm-nucleus not active in fertilization increases but little in size, and its network becomes less dense, resembling that of the conjugating nuclei; it may pass through the ordinary processes of disintegration; and in a few cases, not sketched for lack of space, it has been observed to divide amitotically as described by Arnoldi ('00) in *Cephalotaxus*.

But frequently the sperm-nucleus and occasionally the vegetative nucleus attempt to divide mitotically. One or two small, abortive, karyokinetic figures are not uncommon in the upper part of the egg at the time of the division of the two segmentation-nuclei. I have said, 'attempt to divide,' for no instance has been observed in which the division of these nuclei has extended beyond a late prophase. A bipolar spindle, with the chromatic segments scattered irregularly upon it, represents the most advanced stage which has been seen in the division of the smaller sperm-nucleus (Fig. 87 *b*). (A rupture was made during sectioning in the cytoplasm at one end of this spindle so that the upper pole has been separated into two.) The stalk-cell still persists at this late date (Fig. 87 *b*), and in another section of the series a second mitotic figure appears (Fig. 87). This evidently represents the vegetative nucleus. The achromatic part of the figure presents the appearance of a normal bipolar spindle; but the chromatic spirem has not become homogeneous, and probably would not have developed further. In some cases a well-developed spirem is formed in the upper part of the egg, but no achromatic threads become apparent (Fig. 85); again,

a nucleus seems to have been entirely resolved, during its disintegration, into achromatic fibres. As above stated, in no case observed did the division of these nuclei reach telokinesis; but at some point in the development prior to such a late stage, activity ceased, and disintegration of the nuclear elements took place.

It might be suggested that these division-figures result from the conjugation of the nucleus of the ventral canal-cell with the smaller sperm-nucleus. There is no evidence that such is the case, and I am convinced that they could not have had such an origin. In an examination of many hundred archegonia just before fertilization, no ventral canal-cell containing a normal nucleus has been observed. Shall we, then, conclude that, in a far less number of preparations representing stages immediately following fecundation, fifty or more instances occur in which the nucleus of the ventral canal-cell has conjugated with another nucleus and subsequently divided?

It is generally recognized, especially by cytologists on the animal side, that the stimulus to division is given, not by the egg-nucleus, but by the cytoplasm of the egg. If this be true, it is not strange that these nuclei, lying in a position where everything is most favourable for growth and development—in a medium not only rich in nutritive substances but especially adapted to incite activity in nuclei—should divide. It is a well-known fact that when several spermatozoa enter the ovum of certain animals, only one unites with the egg-nucleus, the others degenerate, or, as is frequently the case, they divide mitotically. And herein we find a further similarity between the processes attending fertilization in some animals and those taking place within the oosphere of *Pinus*.

SUMMARY.

The time at which the archegonia appear, in the species of *Pinus* which I have studied, varies somewhat, but in general they can first be detected about two weeks before fertilization. They are normally found at the micropylar end of the prothallium, and arise by the differentiation of certain of the peripheral cells. By the later growth of the female gametophyte, the mature egg is sunk to a considerable depth in the prothallial tissue, but there always remains an open channel leading from the neck-cells to the nucellar cap. The number of archegonia varies in the different species from one to nine. When the number of oospheres formed is small, they are almost spherical in outline; but this shape may be greatly modified according to the number and arrangement of the archegonia.

In *Pinus Strobos*, the typical neck of the archegonium consists of four cells, all lying in the same plane, while in *Pinus austriaca* and *P. rigida* it is made up of eight, disposed in two layers of four cells each; but there is a lack of uniformity both in the number and in the arrangement of these cells, not only in different, but in the same species.

The central cell is very vacuolate at first, its nucleus always remains close beneath the neck-cells, and is more or less concave on the side toward those cells. When the ventral canal-cell is cut off, about a week before fertilization, the vacuoles have nearly disappeared from the venter of the archegonium.

The spindle in the division of the central cell arises as a multipolar diarch one, and lies wholly within the nucleus. That portion of the mitotic figure which gives rise to the ventral canal-cell varies much in the later stages of its development; but, whatever irregularity characterizes the upper part of this spindle, it always becomes monopolar, or nearly so, at its lower, inner extremity.

The form and structure of the nucleus of the ventral canal-cell are very variable, and are correlated with the irregularities

occurring in the upper, outer portion of the achromatic spindle during the division of the central cell. There are probably instances in which no membrane is developed about this nucleus; in such cases the chromosomes never fuse to form a network. The ventral canal-cell rarely presents the appearance of a normal cell; at the time of fertilization it usually persists as a small, somewhat crescent-shaped, deeply staining body, which lies just beneath the neck-cells of the archegonium and above, but in contact with, the cytoplasm of the egg.

During the maturation of the egg, many nutritive spheres arise in its cytoplasm. At first these are irregularly scattered throughout the cell, though more prominent at its periphery; in the mature egg, they are largely confined to the peripheral portions of the lower half of the cytoplasm. It is suggested, though not definitely demonstrated, that these nutritive spheres are the products of nucleolar activity, having originated within the nucleolus of the egg and the nucleoli of the sheath-cells.

As the egg-nucleus assumes its central position in the oosphere, it increases much in size, and many fibres arise in the cytoplasm surrounding it. These threads have, in general, a radial arrangement and are most prominent along the upper side of the nucleus. The structure presented by the growing, and also by the mature, egg-nucleus may vary from a most delicate network bearing minute granules, to an interrupted, imperfect reticulum composed of large, irregular, diffusely-staining elements. These various aspects are doubtless the expressions of the different physiological activities with which this nucleus is concerned. The normal egg-nucleus has one large, vacuolate nucleolus and a variable number of small, secondary nucleoli. There is no evidence of the presence in this nucleus of a special metaplastic substance.

The egg-cytoplasm presents a delicate reticulum, in which, at times, many fibres occur. Immediately preceding fertilization, an opening arises in this cytoplasm, just below, or in the near vicinity of, the neck-cells. This cavity is apparently formed for the reception of the sperm-cell.

At the time of fertilization, an opening is formed in the apex of the pollen-tube, and the cells of the male gametophyte which still persist, together with a portion of the cytoplasm and some of the starch of the pollen-tube, pass into the cytoplasm of the egg.

The larger sperm-nucleus escapes from the protoplasm of the sperm-cell and moves directly toward the egg-nucleus; the other nuclei from the pollen-tube may persist, in a modified form, in the upper part of the archegonium until the eight-celled stage of the proembryo; but the cytoplasm of the sperm-cell fuses at once with that of the oosphere. The stalk-cell gradually disintegrates and blends with the egg-cytoplasm. The vegetative nucleus and the smaller sperm-nucleus may share the fate of the stalk-cell, but, during the second division following fertilization, they not infrequently give rise to mitotic figures. The smaller sperm-nucleus, then, may pass through a slow process of disintegration, it may divide amitotically, or it may give rise to a karyokinetic figure of more or less definiteness.

There is no apparent change in the diameter of the sperm-nucleus after its entrance into the oosphere. At the time of conjugation, the egg-nucleus is several times larger than the sperm-nucleus, and the sperm-nucleus does not increase in size after its contact with the egg-nucleus. The inequality in the size of the sexual nuclei may be due to the difference in the size of their cells. But if, as has been suggested, the egg-nucleus functions as a manufacturer of nutritive material, may we not find in this activity a feasible explanation of its greater size? The conjugating nuclei always dissimilar in size may, or may not, be similar in structure.

The egg-nucleus becomes slightly concave on the side nearest to the approaching sperm-nucleus. This nucleus embeds itself in the side of the egg-nucleus but does not penetrate its membrane. There is never any fusion, as ordinarily understood, of these two nuclei. A chromatic spirem arises, and a prominent achromatic reticulum becomes apparent in each nucleus. Soon afterwards the nuclear mem-

branes entirely disappear. The two chromatic groups remain distinct until the nuclear plate stage.

The spindle of the first division following fecundation always lies between the conjugating nuclei and parallel with the outer, free surface of the sperm-nucleus. It is multipolar in origin and is probably derived equally from the paternal and the maternal nucleus. The spindle-fibres appear to arise by a rearrangement of the achromatic nuclear reticula and are evidently not the expression of a special kinoplasmic substance. After the formation of the daughter-nuclei, the greater portion, if not all, of these threads pass into the cytoplasmic network. During metakinesis and later stages this spindle may vary from a broad, multipolar diarch to a slender bipolar spindle. The chromosomes pass to the poles in the form of narrow U's.

No individualized centrosomes or centrospheres have been found to occur in connexion with the first division following fertilization. But the entire activity connected with this mitosis indicates that the sperm-nucleus, under the influence of the egg-cytoplasm, is the agent which initiates and controls the division.

The two segmentation-nuclei present a reticulated structure in which the paternal and the maternal chromatin appear to be completely fused. They divide in the upper part of the egg, passing through practically the same steps as those noted for the first division. The two chromatic spirems of each nucleus take up a position along the adjacent sides of the nuclei. A longitudinal splitting of the chromosomes first becomes apparent during an early stage in metakinesis.

The four segmentation-nuclei attain full size while still in the upper part of the egg. As they pass to the base of the oosphere, fibres occur in the cytoplasm similar to the threads observed around the descending egg-nucleus. The steps in the division of these nuclei have not been carefully traced, but, from the stages observed, it is probable that this mitosis does not differ from the division of the two segmentation-nuclei.

No cell-wall is laid down at the base of the oosphere, in the species of pines which we have studied, until after the eight-

celled stage of the proembryo has been reached. These eight nuclei are surrounded by a deeply staining substance which extends out from each nucleus in irregular strands. This substance finally comes to lie in the lines of the future cell-walls, and is evidently transformed into cell-wall.

The number of chromosomes in the nucleus of the ventral canal-cell, in the nuclei of the sheath-cells, and in the egg-nucleus has been found to be 12, while the mitotic figure, in the first division following fertilization, shows 24 chromatic segments.

It is interesting to note the many points of similarity between fertilization as it has been observed in *Pinus*, and the processes known to take place during fertilization in some animals. (1) The egg in *Pinus* is very large and is abundantly supplied with nutritive spheres. (2) The sexual nuclei do not fuse, and no structure which could properly be called a segmentation-nucleus is ever formed. (3) An achromatic nuclear recticulum becomes very prominent in the sexual nuclei during the prophase of division. (4) The chromatin of the sexual nuclei forms two definite groups which remain distinct until metaphase. (5) Two chromatic groups, representing respectively the paternal and the maternal chromatin, appear in the second division following fecundation; and the indications are that they will again occur in the third division and perhaps are characteristic of all the divisions which take place within the oosphere. (6) The nuclei, which enter the egg from the pollen-tube but play no part in fertilization, show a tendency to divide mitotically.

I am pleased to express my gratitude to Professor George F. Atkinson for his most helpful advice and encouragement, and his never-failing kindness, throughout the entire progress of these studies.

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EXPLANATION OF FIGURES IN PLATES XXIII,
XXIV, AND XXV.

Illustrating Miss Ferguson's paper on Fertilization in *Pinus Strobos*.

All figures were drawn with the aid of the Abbé camera lucida, projection 14.3 cm. A Zeiss microscope was used, the lenses being combined as follows: Figs. 8-12, 41, 75-82 with ocular 4 and 16 mm. objective; Figs. 1-7, 38-40, 42-50 *b*, with comp. ocular 12 and objective as before; Fig. 33 with ocular 4 and 4 mm. objective; Figs. 13-32, 34-36, 51-74, 77 *b*, 78 *b*, 79 *b*, 80 *b*, 81 *b*, and 82 *b* with ocular 4 and 2 mm. homo. immer. objective; Figs. 37 *a*-37 *g*, and 88-90 with comp. ocular 12 and 2 mm. homo. immer. objective. The amount of magnification accompanies the description of each figure. The drawings were reproduced without reduction. The lettering of the figures is to be interpreted thus: prothallium (*pr.*), ventral canal-cell (*v. c.*), neck-cells (*n. c.*), egg-nucleus (*e. n.*), sperm-nucleus (*s. n.*), sperm-cytoplasm (*s. c.*), vegetative nucleus (*v. n.*), stalk-cell (*st. c.*), starch (*sh.*), cytoplasm from pollen-tube (*c. p. t.*), nutritive spheres (*n. s.*), primary nucleolus (*py. ns.*), secondary nucleolus (*sy. ns.*).

All the figures, with the exception of Figs. 33 and 80 *b*, have been given their normal position, as nearly as it was possible to do so, on the plates, i. e. they are so placed that the primary axis of the ovule would be parallel with longer axis of the half-plates; and the portion of a figure nearest to the micropylar end of the ovule is always toward the top of the plate. Unless otherwise stated the figures represent stages in the development of *Pinus Strobos*.

PLATE XXIII.

Figs. 1-7. Stages in the early development of the archegonium. $\times 160$.

Figs. 8-11. Later stages in the growth of the archegonium. $\times 70$.

Fig. 12. Mature archegonium. $\times 70$.

Fig. 13. Nucleus of the central cell shortly before its division. $\times 540$.

Figs. 14-18. Prophases in the division of the central cell. $\times 540$. Nos. 16 and 18, *Pinus austriaca*.

Fig. 19. Cross-section of the ventral canal-cell soon after segmentation of the chromatic spirem. $\times 540$.

Figs. 20-21. Disappearance of the nuclear membrane and establishment of the achromatic spindle. $\times 540$.

Figs. 22-29. Separation of the half-chromosomes and formation of the daughter-nuclei. $\times 540$. Nos. 24 and 29, *Pinus austriaca*. These figures show some of the variations occurring in the mitotic figure for this division, and the corresponding variations in the structure of the nucleus of the ventral canal-cell.

Figs. 30-32. Later history of the ventral canal-cell and early stages in the development of the egg-nucleus. $\times 540$.

Fig. 33. Division of the central cell, showing also the lower portion of a pollen-tube which has already reached the endosperm. $\times 230$. *Pinus montana*, var. *uncinata*.

Fig. 88. The primary nucleolus from a mature egg-nucleus with secondary nucleoli clustered about it, and evidently being formed by it. The primary nucleolus has a great affinity for stains at this time. $\times 1200$.

Fig. 89. The framework of the primary nucleolus from a mature egg-nucleus. $\times 1200$. This nucleolus has remained of a light greenish-yellow colour after treatment with Flemming's triple stain.

Fig. 90. The primary nucleolus of a mature egg-nucleus. This nucleolus shows a weak reaction towards dyes, and apparently has an outer limiting membrane. $\times 1200$.

PLATE XXIV.

Figs. 34-35. Later stages in the downward movement and growth of the egg-nucleus. $\times 540$.

Fig. 36. Mature egg-nucleus. $\times 540$.

Fig. 37, *a-g*. Portions of the reticulum from different mature egg-nuclei, showing the variations which may exist in the structure of this nucleus. $\times 1200$.

Fig. 38. The upper part of an archegonium, showing cavity formed in the cytoplasm just prior to fertilization. $\times 160$.

Fig. 39. The upper part of an archegonium just after the entrance into the egg of the elements from the pollen-tube. $\times 160$.

Fig. 40. A slightly later stage. The cytoplasm of the sperm-cell has already fused with the cytoplasm of the egg. $\times 160$.

Fig. 41. An entire archegonium showing the sexual nuclei in contact, and, above them, the various elements which have come into the egg from the pollen-tube. $\times 70$.

Fig. 42. The upper part of an archegonium in the same stage as the above. $\times 160$.

Fig. 43. The sexual nuclei just before coming into contact. Note depression in egg-nucleus. $\times 160$.

Figs. 44-50. Various appearances presented by the conjugating nuclei. It will be borne in mind that these figures are so placed that the major axis of the archegonia in which they occur would lie parallel with the longer axis of the half plate. $\times 160$.

Fig. 50 *b*. Another section through the egg-nucleus shown in number 50. There is a greater difference in the size of the conjugating nuclei than would appear in figure 50. $\times 160$.

Fig. 51. An early prophase in the first division following fecundation. $\times 540$.

Fig. 52. A slightly later stage. The cytoplasm caught between the two nuclei has collected into spherical masses. $\times 540$.

Fig. 53. A still later stage in the formation of the two chromatic spirems. $\times 540$.

Fig. 54. A still later stage in which the paternal chromatic spirem has taken up a position near the maternal spirem, and a few delicate achromatic threads have made their appearance in the neighbourhood of these spirems. The nuclear membranes are still present, but have broken down at several points. $\times 540$.

Fig. 55. A later stage. The nuclear membrane has entirely disappeared; the spindle fibres have increased in number; and the rearrangement of the achromatic, nuclear reticula into granular threads is very apparent. $\times 540$.

Figs. 56-57. More advanced stages in the formation of the spindle. $\times 540$.

PLATE XXV.

Fig. 58. The spindle fully established; the two chromatic spirems still perfectly distinct. $\times 540$.

Fig. 59. The two spirems after segmentation; the two halves of the spindle seem to indicate the maternal and the paternal portions of the mitotic figure. $\times 540$.

Fig. 60. Early stage in the formation of the chromosomes. The chromatic elements still occur in two distinct groups. $\times 540$.

Fig. 61. The chromosomes being oriented at the nuclear plate. The distinction between paternal and maternal elements no longer evident. $\times 540$.

Fig. 62. A cross-section through the nuclear plate just before the splitting of the chromosomes; twenty-four segments are distinctly shown. $\times 540$.

Figs. 63-65. Some of the aspects presented by this mitotic figure during meta-kinesis. $\times 540$.

Fig. 66. An anaphase of the mitosis. .

Fig. 67. A late anaphase of the division; the poles terminate in granular areas from which delicate threads extend into the cytoplasm; some of the nucleolar substance from the egg-nucleus still persists. $\times 540$.

Fig. 68. One end of a spindle in the same stage as the above; the fibres which radiate from the polar region of the spindle are very abundant and stain deeply. $\times 540$.

Fig. 69. One aspect presented by the karyokinetic figure in the telophase of this division. $\times 540$.

Fig. 70. The two segmentation-nuclei fully formed. $\times 540$.

Fig. 71. One of the two segmentation-nuclei in an early prophase of division. $\times 540$.

Figs. 72-73 *b*. Later stages in the second division, showing two chromatic spirems. $\times 540$.

Fig. 74. A still later stage. $\times 540$.

Fig. 75. An entire archegonium showing the position of the two segmentation-nuclei during division. $\times 70$.

Fig. 76. An archegonium showing the original position of the four segmentation-nuclei. $\times 70$.

Fig. 77. The same after the nuclei have begun their downward movement. $\times 70$.

Fig. 78. The same after the nuclei have almost reached the base of the oosphere. $\times 70$.

Fig. 78 *b*. A portion of number 78, showing details in nuclear structure, and fibres in the surrounding cytoplasm. $\times 540$.

Fig. 79. The lower part of an archegonium after the four nuclei have arranged themselves at the 'organic apex' of the oosphere. $\times 70$.

Fig. 79 *b*. A portion of the above; the nucleus is in the early prophase of division; the cytoplasm surrounding the nucleus has become dense and deeply staining. $\times 540$.

Fig. 80. The basal portion of an egg; the four segmentation-nuclei are in a metaphase of the mitosis. $\times 70$.

Fig. 80 *b*. A part of the same showing details. $\times 540$.

Fig. 81. A portion of the lower part of an oosphere after the formation of the eight nuclei of the proembryo. $\times 70$.

Fig. 81 *b*. A part of the above giving details. No cell-walls have as yet been formed. $\times 540$.

Fig. 82. A somewhat later stage than number 81. $\times 70$.

Fig. 82 *b*. A portion of the above, showing cell-walls in the process of formation. $\times 540$.

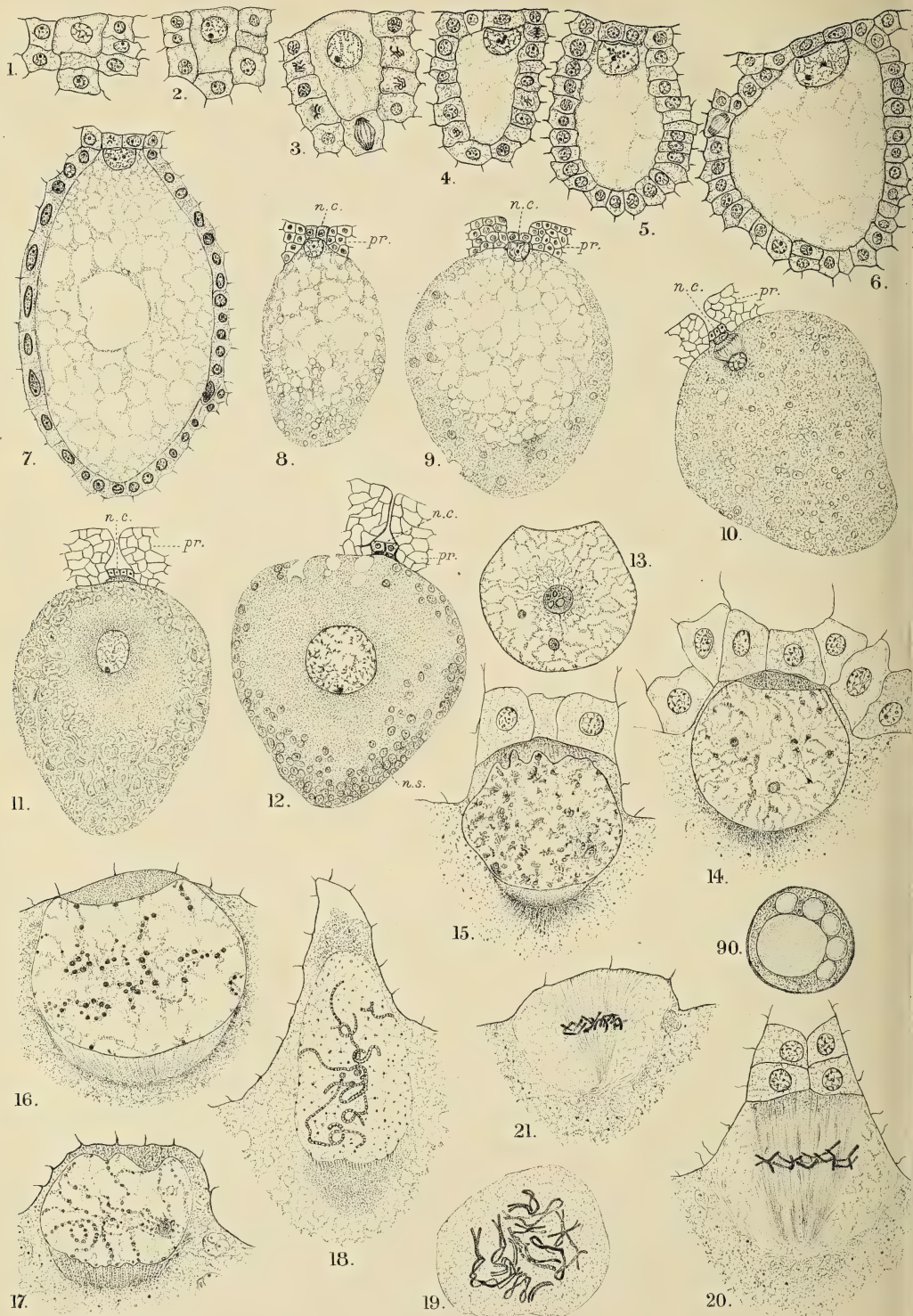
Figs. 83-84. Some of the aspects presented by the ventral canal-cell. $\times 540$.

Figs. 85-86. Figures occurring in the upper part of archegonia during the division of the segmentation-nuclei. $\times 540$.

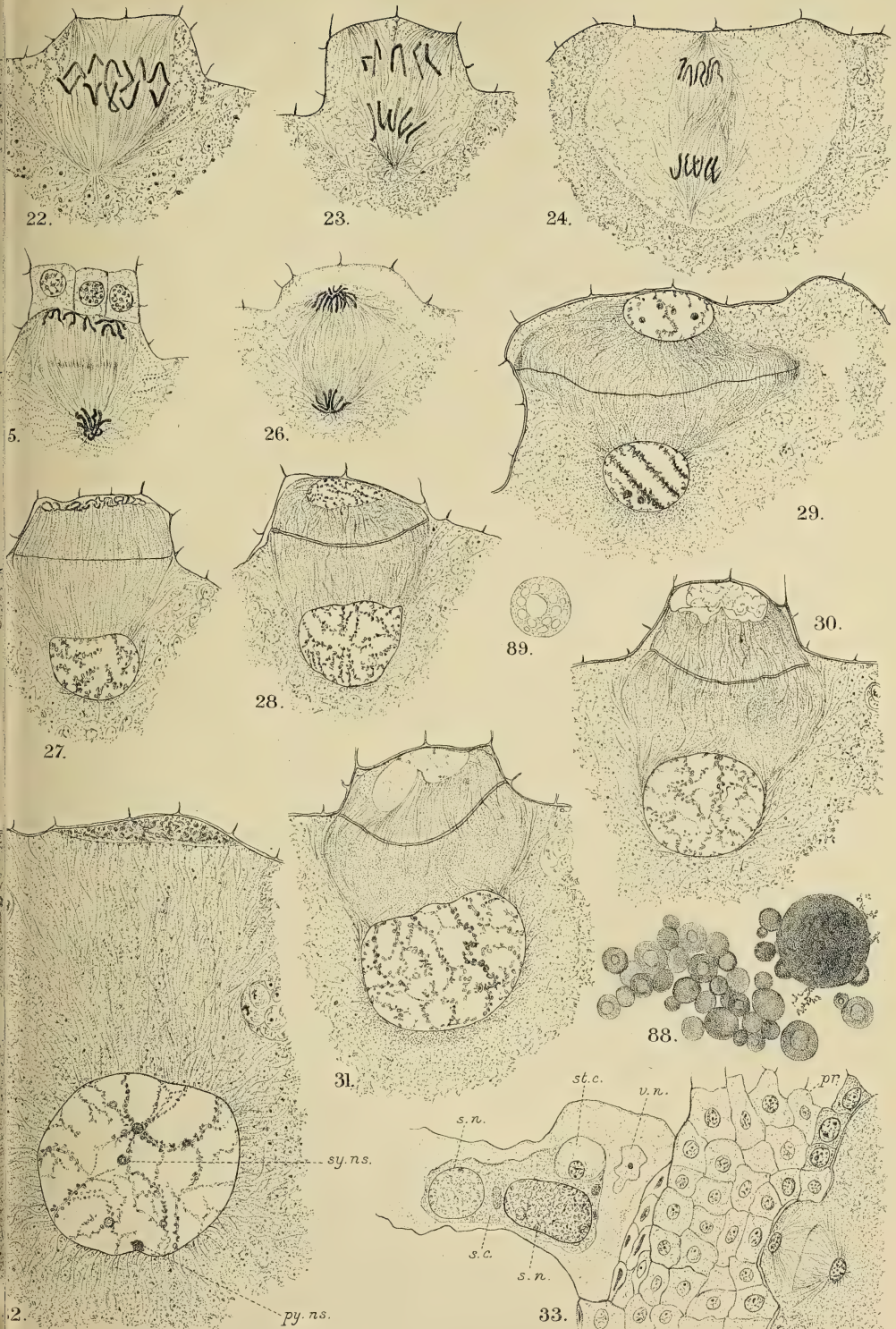
Figs. 87-87 *b*. Figures occurring in the upper part of an archegonium at the time of the second division following fertilization: Fig. 87 represents the vegetative nucleus, and the karyokinetic structure in Fig. 87 *b* the smaller sperm-nucleus; just above this figure is the stalk-cell. $\times 540$.

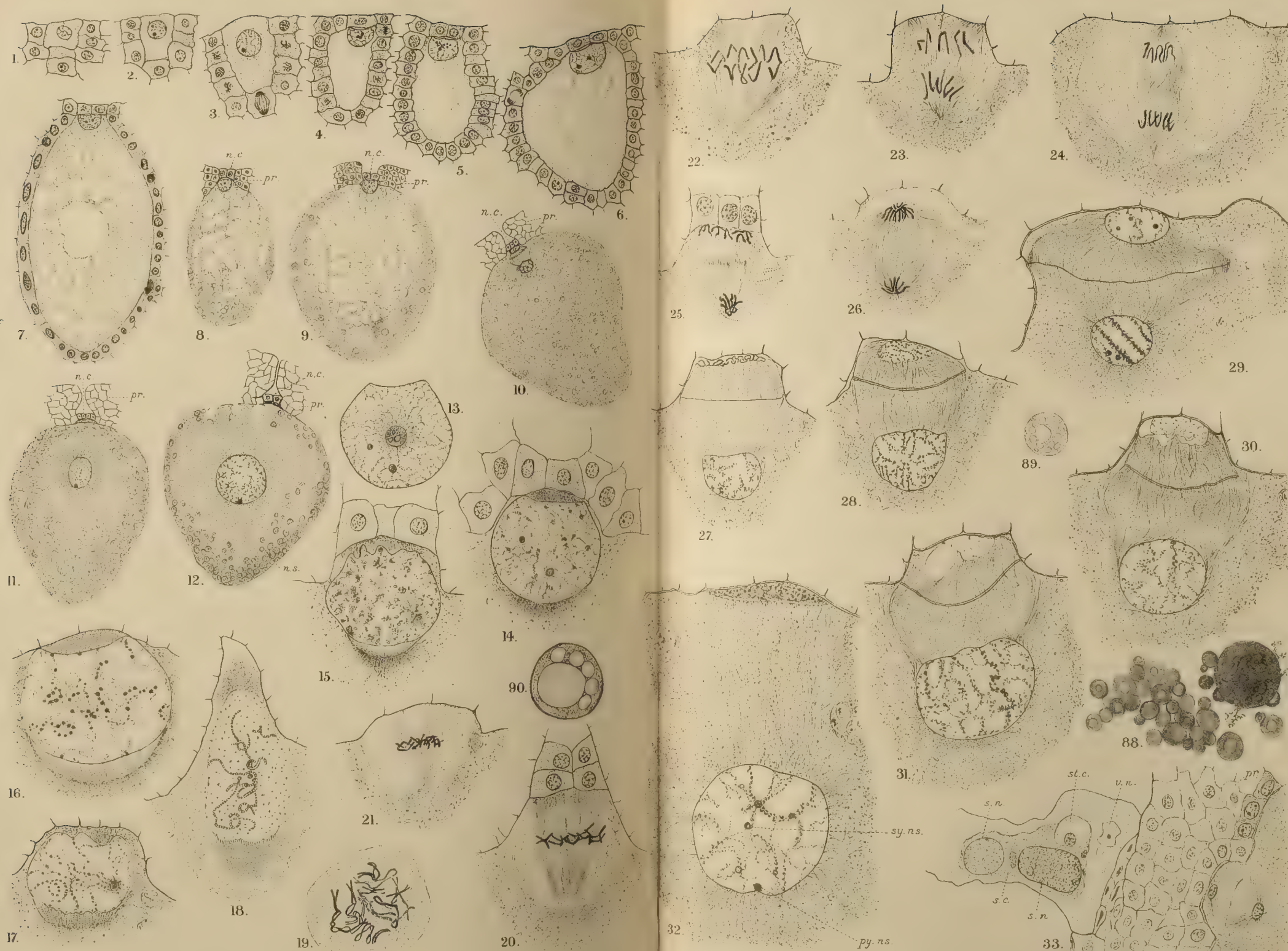
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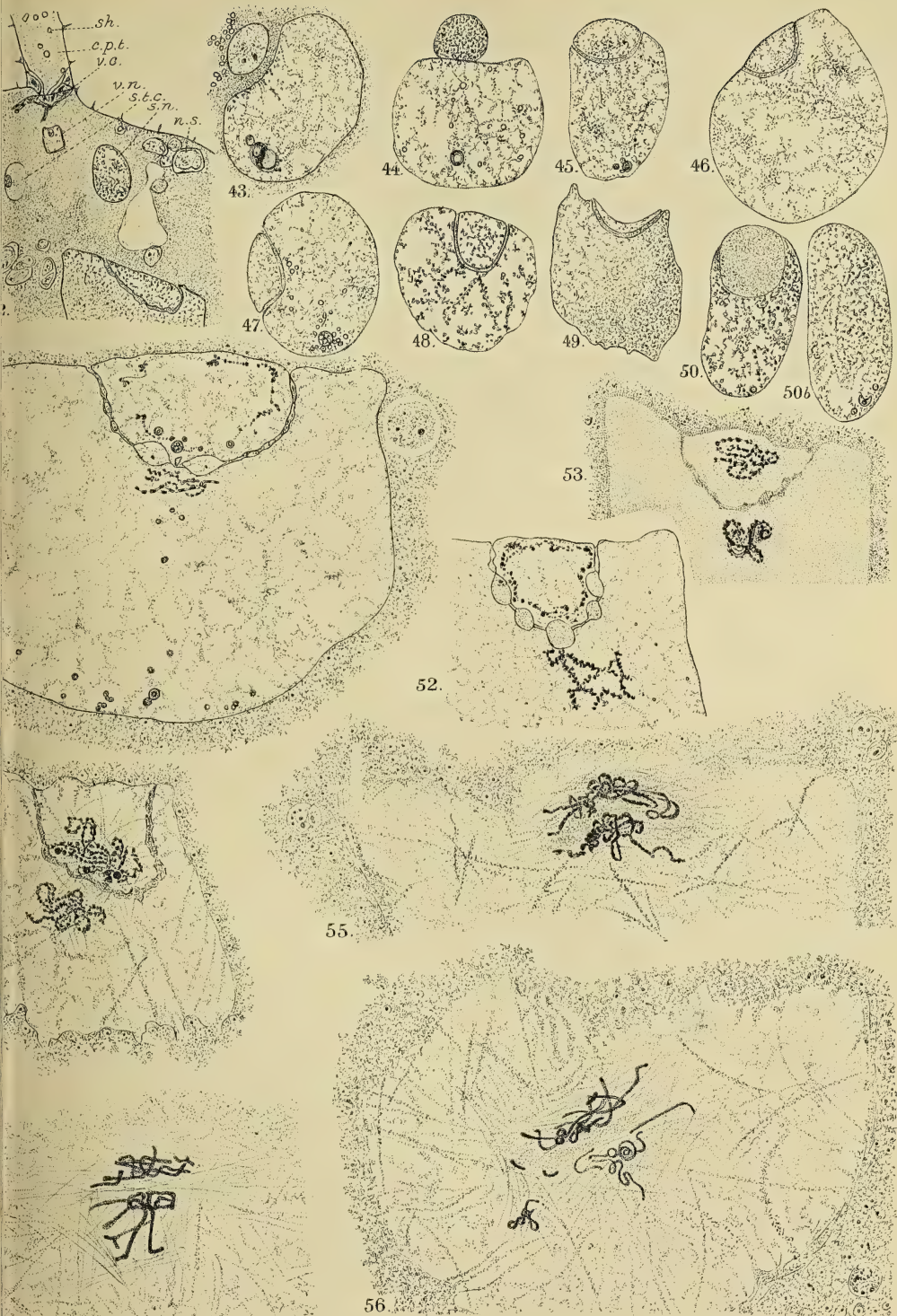


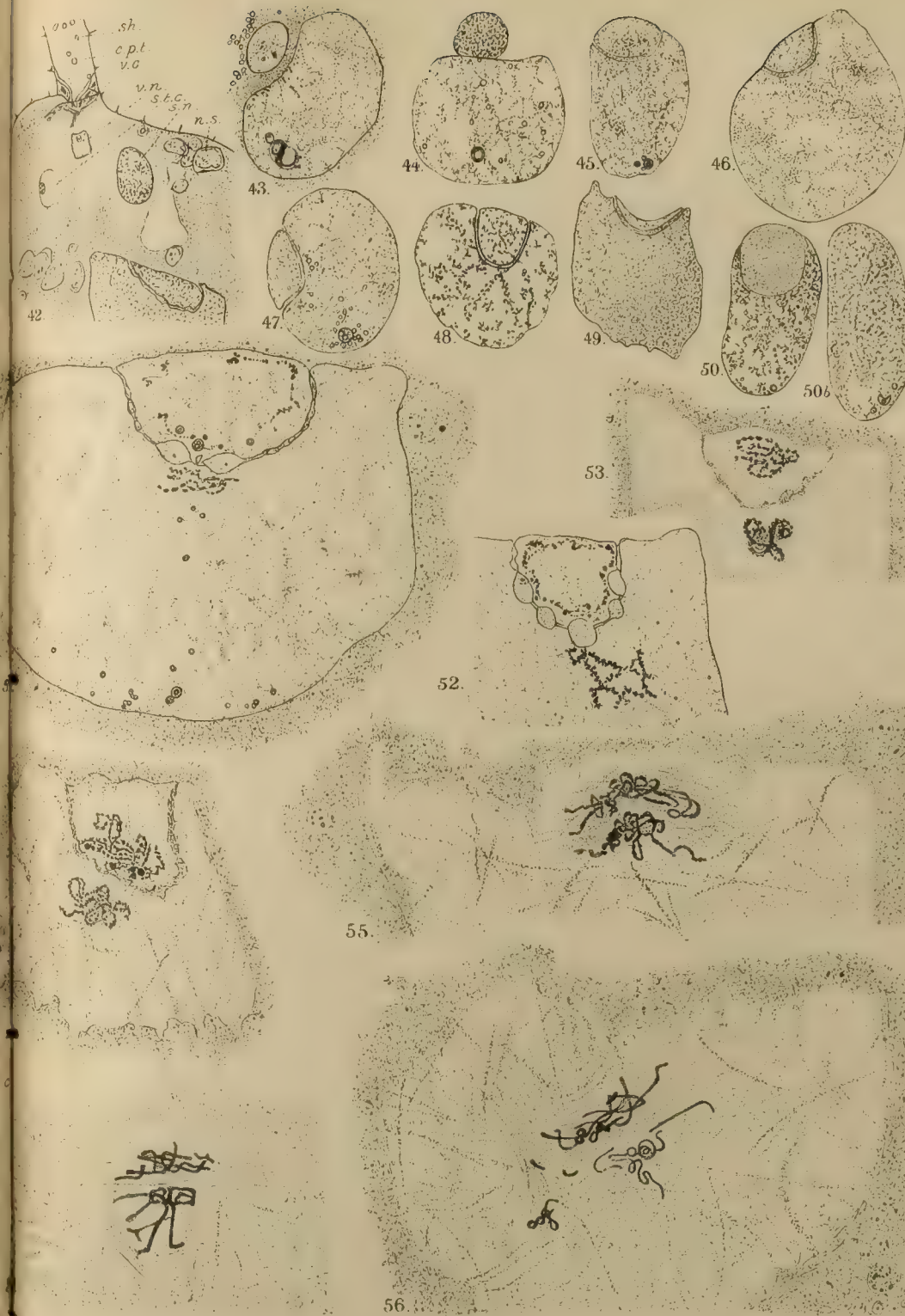


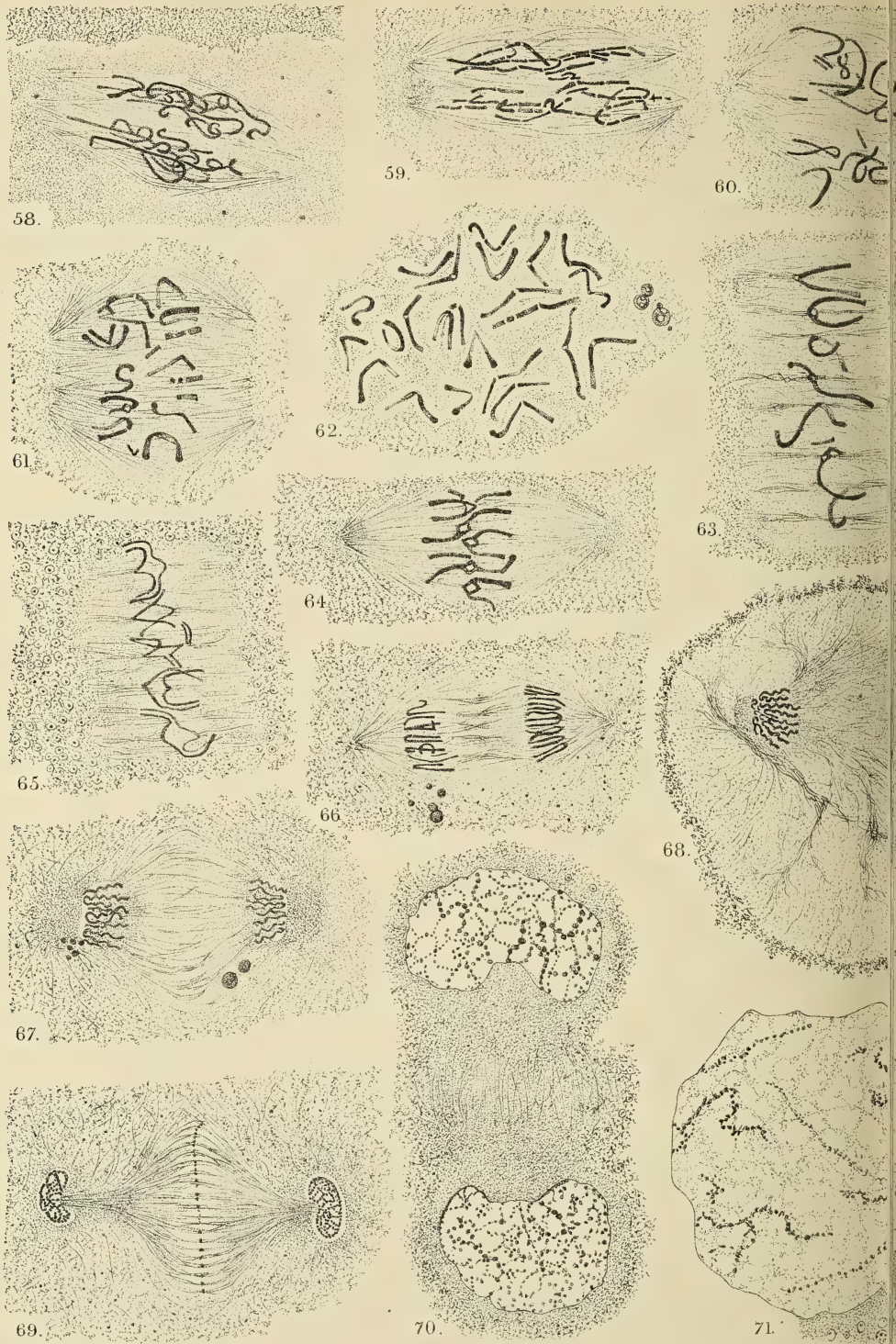
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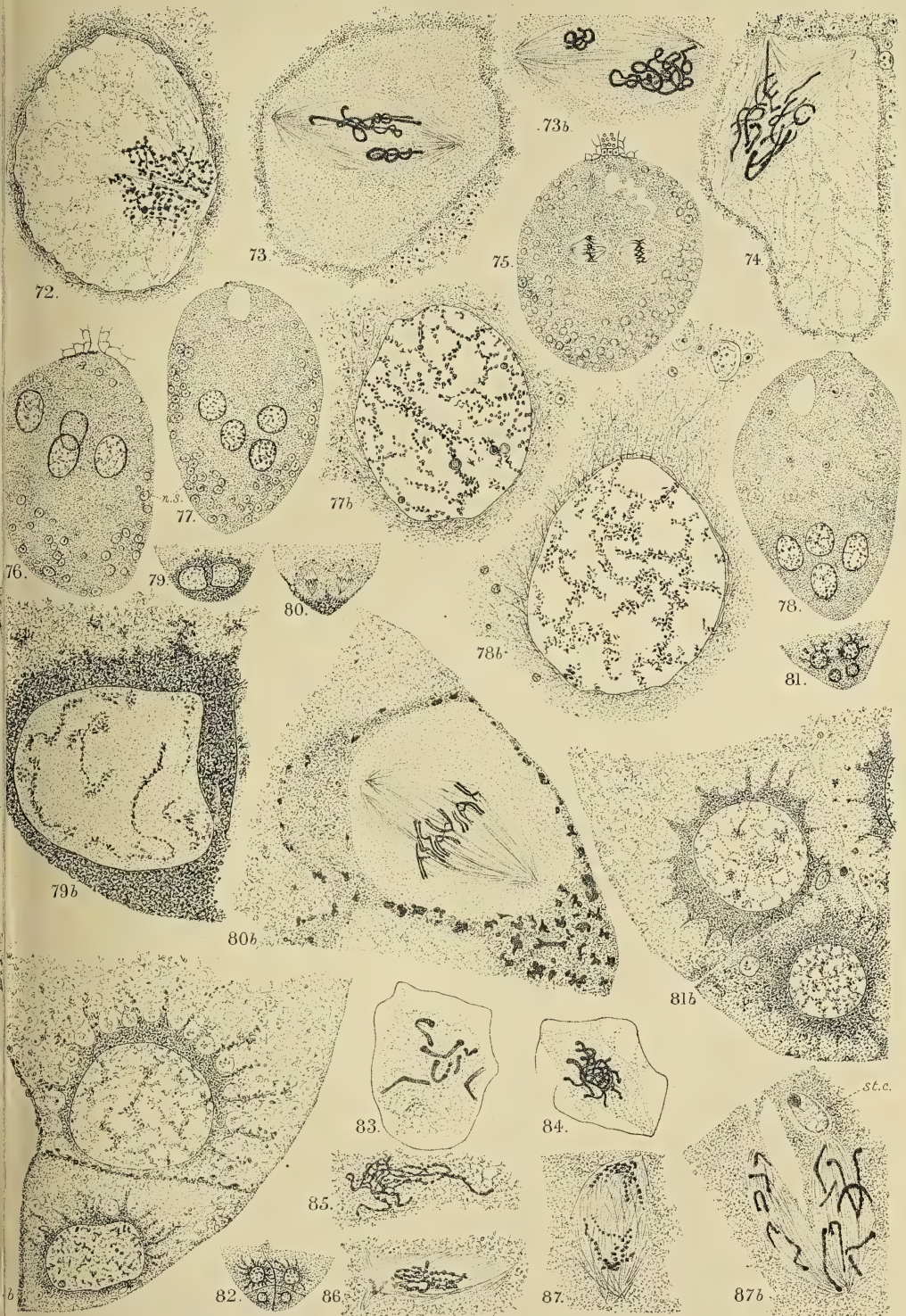
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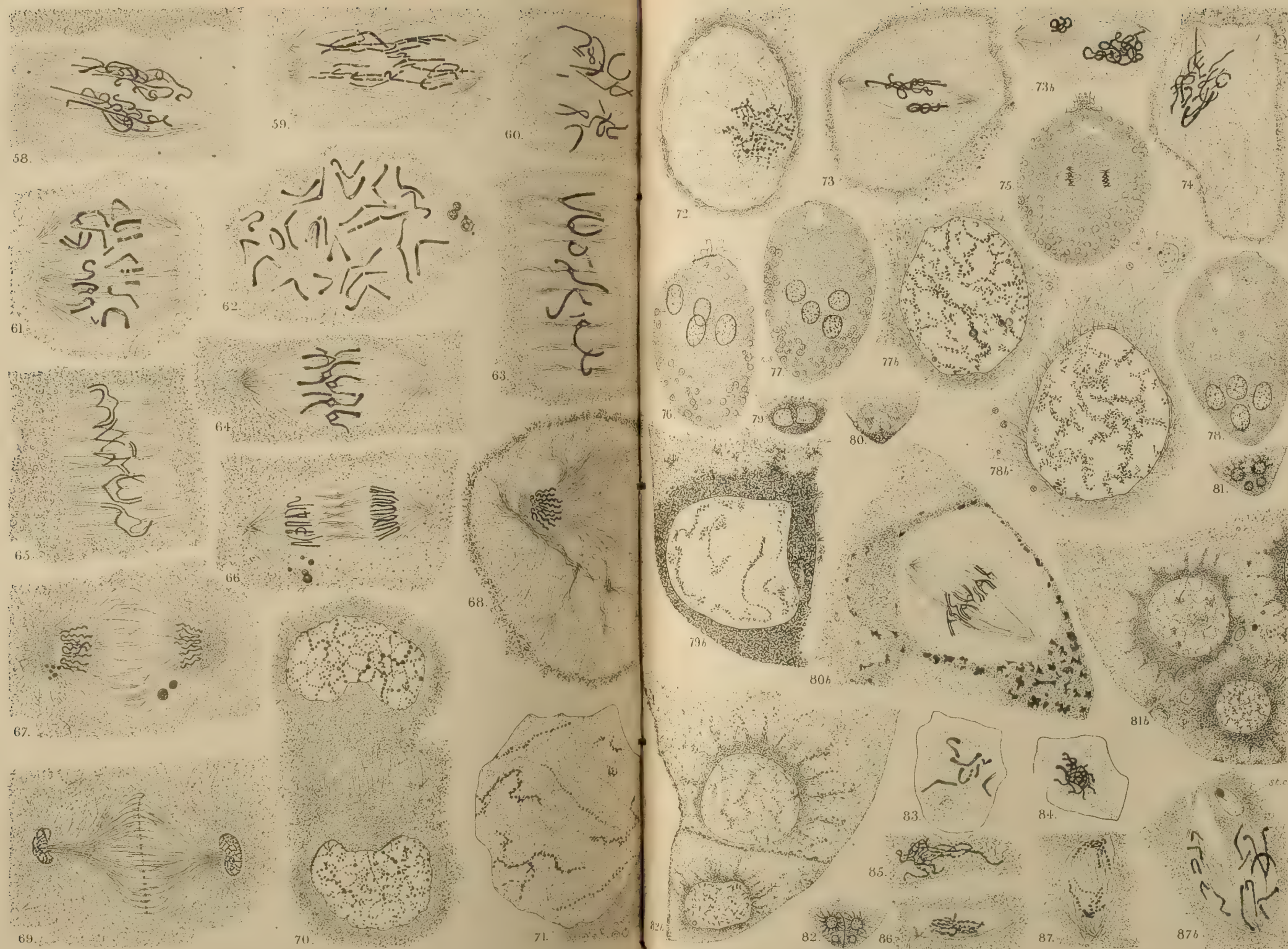






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FERGUSON — FERTILIZATION IN PINUS STROBUS.

University Press, Oxford.

Note on Phyllotaxis.

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With two Figures in the Text.



WRITERS on Phyllotaxis are generally agreed in accepting the series of formulae known as the Schimper-Braun series of divergences, $\frac{2}{5}$, $\frac{3}{8}$, $\frac{5}{13}$, &c., as fundamental expressions of the primary phenomena of the arrangement of lateral members. This series of fractional expressions, which involves the utilization of the Fibonacci ratio series 2, 3, 5, 8, 13, &c., has thus proved for over sixty years the ground-work of all theories of phyllotaxis, and is usually described in the early pages of textbooks. Taking the ' $\frac{2}{5}$ ' as a type of these values, this expression implies that in placing five members on a spiral which makes two complete revolutions of an axis, the sixth member is mathematically superposed to the first, and that successive members differ by a divergence-angle of 144° . So simple are these relations and so thoroughly well known that it is not necessary to dwell further on the vast superstructure of morphological theory which has been built up on this foundation. However, as a matter of fact, taking the $\frac{2}{5}$ divergence again as an example, it is beyond doubt that observation of the actual plant shows that these relations do not strictly hold, and various theories

have at different times been proposed to show why this should be so; these again agree in taking the fractional expressions as representative of some mathematical law, all deviations from which must be due to the action of secondary forces, real or hypothetical. Such speculations include the original prosenthesis theory of Schimper and Braun, various torsion and displacement theories, culminating in the contact-pressure theory of Schwendener. These various views have been recently critically examined by Winkler (Pringsh. Jahrb., 1901, Heft I).

Since the general plan of these investigations consists, however, in superimposing some new hypothesis on the original conception of Schimper and Braun, a strict analysis of the subject demands a preliminary investigation of the views of Schimper and Braun and the scientific evidence underlying these fractional expressions, which become translated into accurate divergence-angles of degrees, minutes, and seconds. So long have these numbers been accepted that it appears somewhat gratuitous to point out that these generalizations rest on no scientific basis whatever, and that what passed for evidence in 1830 does not necessarily hold at the present day. Thus Schimper and Braun elaborated these expressions of divergence on the plan of the original $\frac{2}{5}$ or *quincuncial* system proposed by Bonnet in 1754. The starting-point in dealing with phyllotaxis is therefore the elucidation of the exact point of view of Bonnet, which has determined the path along which all subsequent investigation has proceeded. Now Bonnet, who had the assistance of the mathematician Calandrini, studied adult axes only, and devised, as an expression of the facts observed on *elongated* leafy shoots, a helix winding round a cylinder and spacing out at equal angles five members in two complete revolutions, the sixth member falling on the same vertical line as the first; a simple mathematical conception was thus utilized to express the observed phenomena. The fact which Bonnet thoroughly understood, that on a plant-shoot the sixth leaf did *not* fall exactly over the first, but that the series formed by every fifth leaf itself wound along a spiral

path, was explained by an assumption which has exerted a powerful influence on subsequent speculations, that the plant in fact purposely destroyed the postulated mathematical construction, in order that the assimilating members might be given free transpiration-space without any overlapping. Generally speaking, but little real advance has been made in the investigation of the primary causes of phyllotaxis beyond these original views of Bonnet published nearly 150 years ago. It will be noticed that the fractional expressions of Schimper and Braun repeat the hypothesis of Bonnet in a more elaborated form; the Fibonacci series of ratios is introduced in full, but these are so associated as to still imply helices wound on cylindrical axes. However, as pointed out by the brothers Bravais, axes are commonly conical, dome-shaped, or even nearly plane, and on such surfaces the helices would be carried up as spirals of equal screw-thread, and thus become curves which in the last plane case are spirals of Archimedes. That is to say, by expressing the helix-construction in the form of a floral-diagram, the position of leaves being marked on concentric circles whose radii are in arithmetical progression, the genetic spiral becomes a spiral of Archimedes, and the *orthostichies* are true radii vectores of the system. Such a geometrical construction is implied in the Schimper-Braun terminology which postulates the existence of orthostichies as straight lines. At the same time, by drawing curves through the same points in different sequence, other spirals appear in the construction, and these, distinguished as *parastichies*, are similarly by construction spirals of Archimedes.

Such geometrical plans are given in textbooks, and are used for instilling a primary conception of the arrangement of lateral members; the fact that they do not always agree with actual observations is glossed over by the assumption of secondary disturbing agencies, as for example *torsion*.

On examination, these fundamental expressions are seen to be based on:—

1. The assumption of a special divergence-angle.

2. The existence of accurate *orthostichies*: these latter following from the construction as being radii vectores of a spiral of Archimedes, the spiral again being derived from Bonnet's helix with parallel screw-thread.

Since helices and spirals of Archimedes are also commonly the result of torsion-action, the way becomes paved for the addition of theories of lateral displacement or torsion-effects, which are expected to produce secondary alterations in the original simple system of Schimper and Braun.

It becomes therefore necessary to test the basis of these generalizations, and to examine the possibility of checking by direct observation either the divergence-angle or the orthostichies themselves; and finally to compare the plane constructions by spirals of Archimedes and see how far these really do interpret the appearances seen in a transverse section of the developing system in the plant.

Such investigation shows that the hypotheses have no true basis, while the construction by spirals of Archimedes is a conspicuous failure. Thus, the divergence-angle is hopelessly beyond the error of actual observation on the plant, since the points from which the angles have to be taken must be judged by the eye; when, therefore, the divergence-angles are expected to be true to a matter of minutes and seconds in fairly high divergences, this becomes a matter of impossibility; and the Bravais showed in 1835 that it was in fact impossible to *disprove* the standpoint that there was only one angular divergence in such cases of normal Fibonacci phyllotaxis, namely Schimper's 'Ideal Angle' of 137° , $30'$, $27''\cdot936$. Similarly, it is equally impossible to judge straight lines by the eye alone, and the existence of *orthostichies* in spiral phyllotaxis as mathematically straight lines thus becomes as hypothetical as the Schimper-Braun divergence-angles. In neither of the two methods used for the practical determination of phyllotaxis-constants is there then any possibility of accurate mathematical demonstration. Although the tabulation of appearances as judged by the eye may be taken as an approximately accurate version of the real

phenomena, it is clearly impossible to found any modern scientific generalizations on angles which cannot be measured, and lines which cannot be proved to be straight: it thus follows that all speculations based on the assumption of the Schimper-Braun series must rest on a purely hypothetical foundation which may at any time be overturned. Such expressions, as Sachs constantly pointed out, attempt to imitate the phenomena observed without giving any reason for such geometrical construction.

Again, taking the mathematical interpretation of the Schimper-Braun system, that the genetic spiral and the parastichies are represented by spirals of Archimedes, while the orthostichies are radii vectores, a simple geometrical construction in terms of these spirals should bring out either the truth or error of this hypothetical relationship of the lateral members.

Thus, from the equation to the Archimedean spiral ($r = a\theta$), it is easy to construct a pair of spirals whose variable a shall have the ratio of the parastichies observed on any given specimen. Take for example the $\frac{8}{13}$ system, the primary contact parastichies of which are 8 and 13; Fig. 2 shows such a system geometrically planned for a left-hand genetic spiral: the members along the twenty-one orthostichy lines differ by twenty-one, and fall on the mathematically straight radii vectores of the system. The intersections of these parastichy spirals mark the *points* at which the lateral members are inserted, and the views of Schimper and Braun included only the consideration of such points. It is clear, however, that if the spaces between the spiral planes are regarded as containing the members pressed into close lateral contact, as seen in the transverse section of a foliage bud, the appearance of the progressive dorsiventrality of such lateral members is very fairly *imitated*. The construction, in fact, becomes more and more like the appearances seen in the plant as the periphery of the system is reached, but the central part which includes the actual seat of development is very inadequately represented: thus, the areas become so relatively elongated in the

radial direction as they approach the centre that they cannot possibly represent any formation of primordia at the stem-apex, on which such members are well known to arise as fairly isodiametric protuberances. At the same time, it will be noticed that the Archimedean spirals by construction all fall into the centre and stop there, so that no room is left in the

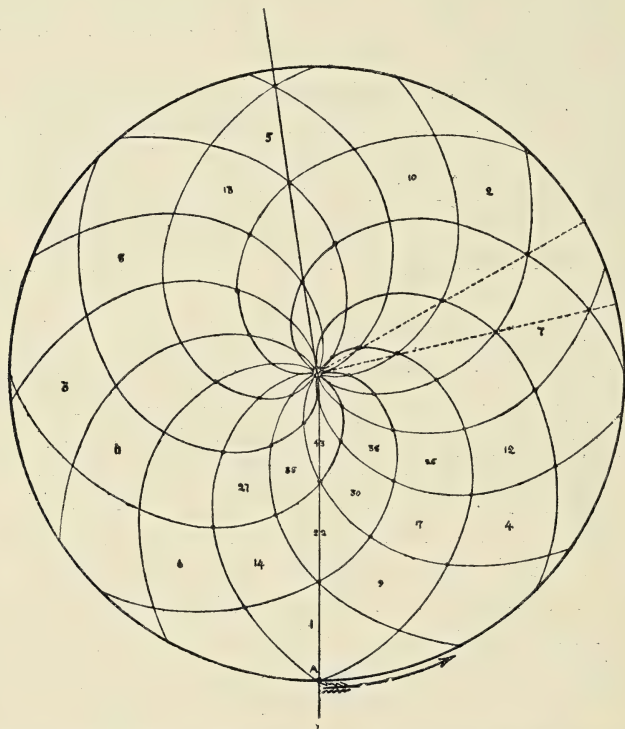


Fig. 2. Theory of Schimper and Braun. Construction for Phyllotaxis $\frac{8}{21}$. OA . = Orthostichy line = radius vector passing through 1, 22, 43, &c. Members along the contact parastichies differ by 8 and 13 respectively. Genetic spiral winds left. Divergence-angle = $\frac{8}{21}$ of $360^\circ = 137^\circ 8' 34''$.

system for any subsequent growth and the addition of new members which naturally obtains in the plant.

Again, further consideration shows that all spirals, whatever their primary nature may have been, must necessarily pass

into Archimedean spirals, which differ by a constant along each radius vector, if they represent the limiting planes of members which grow to a constant bulk and then remain stationary, in the manner that lateral members do on the plant. The appearance of Archimedean spirals on adult shoots is thus secondary, and is merely the expression of the attainment of uniform volume by members in spiral series; it has nothing to do with the facts of actual development, during which lateral members arise as *similar protuberances*, which may be indefinitely produced without the possibility of the system being closed by a terminal member.

In other words, the genetic spiral must be regarded mathematically as *winding to infinity*, and being engaged in the production of *similar members*. That is to say, the possibility is at once suggested that the genetic spiral can only be represented by a *logarithmic* or equiangular spiral which makes equal angles with all radii vectores.

Not only is this a mathematical fact there is no gainsaying, but the introduction of log. spirals into the subject of Phyllotaxis at once opens up wide fields for speculation, in that these spirals are thoroughly familiar to the mathematician and physicist; representing the laws of mathematical asymmetrical growth around a point, they constitute in Hydrodynamics the curves of spiral-vortex movement, while their application to Magnetism was fully investigated by Clerk Maxwell. The possibility that the contact parastichies may be also not only log. spirals but log. spirals which intersect orthogonally, and thus plot out a field of distribution of energy along orthogonally intersecting paths of equal action, is so clearly suggested that it may at once be taken as the groundwork of a theory of phyllotaxis more in accordance with modern lines of thought (cf. Tait, 'Least and Varying Action,' article *Mechanics*, Enc. Brit., vol. 15, p. 723).

A geometrical construction in terms of such spirals in the ratio (8 : 13) (Fig. 3) may be taken as a representative system corresponding to the preceding phyllotaxis-plan of Fig. 2.

It is difficult to avoid the conclusion that the log. spiral

construction gives the true key to the problem, and that the whole subject thus becomes a question of the mechanical distribution of energy within the substance of the protoplasmic mass of the plant-apex: that phyllotaxis phenomena are the result of inherent properties of protoplasm, the energy of life being in fact distributed according to the laws which govern

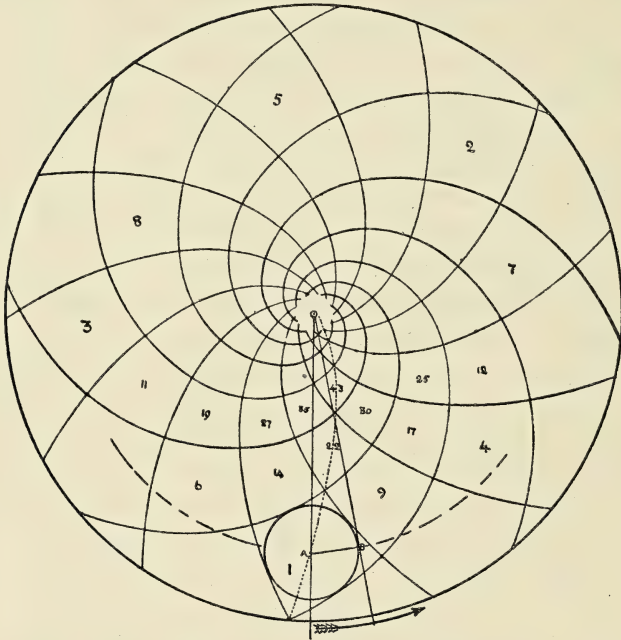


Fig. 3. Log. spiral theory: Construction for Phyllotaxis system (8+13) in terms of distribution of energy. Contact Parastichies = orthogonally intersecting log. spirals in ratio (8 : 13). The curve through 1, 22, 43, &c., is also a log. spiral. Genetic spiral winds left. Divergence-angle = $137^{\circ} 30' 38''$. Bulk-ratio of axis to primordium = $OA, AB. = 1 : 5$ within a small error, or $\sin AOB = .204$ for the true curve.

the distribution of energy in any other form: and that the original orthogonal planes, the relics of which survive in the contact parastichies of the system, represent the natural consequence of a mechanical system of energy-distribution directly comparable with that which produces the orthogonal intersection of cell-walls at the moment of their first formation,

which was deduced by Sachs from the analogy of the orthogonally intersecting planes of thickening observed in cell-walls and starch-grains.

The readiness with which the several problems of phyllotaxis may be solved from this standpoint, when once the key to the whole subject is grasped, is very remarkable, and these views have been elaborated to considerable length in a paper which awaits publication. The results are so varied and striking that it is difficult to give any summary of them in a small space: based as they are on the relative value of the spirals of Archimedes and logarithmic spirals as interpreting the true developmental spiral of the plant-apex, it is evident that the discussion of such curves is beyond the province of the non-mathematical botanist. The object of the present note is therefore merely to point out that the subject of phyllotaxis thus enters entirely new ground which promises results more fundamental than any yet obtained in the domain of plant morphology: for example, it follows in such constructions that an equation may be given for the plane section of a lateral primordium which will serve as a true mathematical definition of a leaf, differentiating it from a stem: the true divergence-angles may be calculated, and a definite numerical value can be given to the ratio $\frac{\text{axis}}{\text{primordium}}$ which determines any given system; while the geometrical constructions, on the plan of Fig. 3, have the advantage that they do agree with the appearances observed in the plant; they obey and amplify Hofmeister's law, and from the standpoint of energy-distribution afford the clue to the subsequent building up of the elaborate '*expansion-systems*' of which the capitulum of *Helianthus* may be taken as a type.

It is not proposed at present to go into further detail as to these questions which are very fully discussed in the paper already prepared for publication; until logarithmic spirals are more familiar to the botanist it will be sufficient to point out that the true key to phyllotaxis is undoubtedly to be found in the solution of the problems of symmetrical or asymmetrical

distribution of energy in orthogonally intersecting planes around an initial 'growth-centre'; in the latter case the whole of the spiral paths are log. spirals. The perfection of such a construction involves uniform growth in the system; and owing to the obvious impairment of this uniform rate of growth behind the plane portion of the apex, the true log-spirals are possibly never to be observed on the plant, although the approximation has been found in certain cases to be extremely close. Ultimately all these curves pass into spirals of Archimedes as the members cease growth on the attainment of constant volume, and these latter curves therefore occur on adult axes and appeal to the eye in the macroscopic view of the entire shoot. They were thus correctly isolated by Bonnet, to whom the detailed construction of the growing point was naturally unknown in 1754. The curves seen in transverse section of an apical system of developing members are thus probably curves transitional between log. spirals and spirals of Archimedes.

On the other hand it will be noted that the new constructions are equally incapable of absolute verification by any angular measurements on the plant; Schimper's orthostichies have vanished, as pointed out by the Bravais, for the more general examples of phyllotaxis, and the difference between the two spiral systems is very slight to the eye: but, while the Schimper-Braun School only sought to imitate the appearances seen on the plant, the log. spiral theory gives at least an equally correct summary of the facts observed, and is in addition founded on definite mechanical laws of construction by orthogonal trajectories which have already been accepted for plant anatomy; it is so far then the logical outcome of Sachs' theory of the orthogonal intersection of cell-walls, and represents therefore another special case of the distribution of energy along planes of equal action¹.

BOTANIC GARDENS, OXFORD.

May, 1901.

¹ Cf. Church, On the Relation of Phyllotaxis to Mechanical Laws. Part I, Construction by Orthogonal Trajectories. 1901.

On the Origin, Development, and Morphological Nature of the aërial Tubers in *Dioscorea sativa*, Linn.

BY

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Dfeiffer Student, Girton College, Cambridge.

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With Plate XXVI.

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THE species *Dioscorea sativa* was founded by Linnaeus in 1753¹, but, according to Bentham², nearly all modern authors have transposed the names of this and another Linnaean species, *D. bulbifera*; perhaps because both produce aërial tubers in the leaf-axils. Hooker³ states that 'the species of *Dioscorea* are in a state of indescribable confusion.' Another source of difficulty in determining the species of *Dioscorea* is the use of the English name 'Yam,' because earlier writers applied it indiscriminately to any edible underground tuber, so that, at first, it included *Batatas* (*Ipomœa*) *edulis* (the sweet potato, one of the Convolvulaceae), *Manihot utilissima* (cassava, one of the Euphorbiaceae) and the aroid *Amorphophallus campanulatus*. Even the potato

¹ Linnaeus, *Species Plantarum*, vol. ii, p. 1033 (1753).

² Bentham, *Flora Australiensis*, vol. vi, p. 462 ('73). *Flora Hongkongensis*, p. 368 ('61).

³ Hooker, *Flora of British India*, vol. vi, p. 288 ('94).

(Solanaceae) has been confused with them, as its name is a corruption of *Batatas*, and was at first applied to the sweet potato, which was introduced into Europe before the plant we now know as potato.

These difficulties render the history of the *Dioscoreas* very obscure. The origin of most of the cultivated forms is either unknown or doubtful. According to the writer of the article on *Dioscorea* in the Dictionary of the Economic Products of India ('90), p. 115 et seq., the existing evidence points to a possible independent origin of the cultivated species of *Dioscorea* in Asia, Africa, and America. The same author considers that the reason why these plants were cultivated for food later than other vegetables is because the wild forms produced edible tubers without cultivation.

All attempts to distinguish between the Linnaean species *D. sativa* and *D. bulbifera*, by referring to original authorities, in order to determine the species upon which the observations in this paper were made, have proved unsuccessful. The characters of the species in question agree with Kunth's detailed description of *Helmia bulbifera*¹ which, according to Hooker², is a synonym of *D. sativa*, Linn., and *D. bulbifera*, Br. Kunth himself, however, regards his *Helmia bulbifera* as identical with the *D. bulbifera* of Linnaeus and Wight. Bentham³ regards *Helmia bulbifera*, Kunth, and *D. bulbifera*, Wight⁴, non-Linn., as synonyms of *D. sativa*, Linn.

D. sativa, Linn., is a widely distributed plant. It grows wild throughout India, and is the species most generally cultivated, so that it is known as 'the common yam.' It is also known in Malabar, Java, the Philippines, Australia, Queensland, and in the West Indies.

References to the tubers in this species are made by the following authors:—

¹ Kunth, Enumeratio Plantarum, vol. v, p. 435 ('50). In the Index Kewensis this is said to be *Dioscorea sativa*.

² Hooker, Flora of British India, vol. vi, p. 295 ('94).

³ Bentham, Flora Hongkongensis, p. 368 ('61).

⁴ Wight, Icones, vol. iii, plate 878.

Hooker¹ says the underground tubers are 'large, and variable in form,' the stem 'bulbiferous.'

Bentham² says, 'stems from a tuberous rhizome, elongated and twining, often bearing green globular bulbs in the axils of the leaves.'

Again he says³, 'Stems glabrous, often bearing green globular bulbs in the axils of the leaves.'

Trimen and Hooker⁴ note that the 'root-tubers are very large, globose or elongate, stem . . . tuberiferous in the leaf-axils.'

The writer of the article in the Dictionary of the Economic Products of India does not mention the tubers of *D. sativa*, but he notes that in Malabar and Travancore there are two species which bear, on the stems, tubers which are ovate in shape, and which vary in size from about that of a pea, to three inches in diameter. These axillary tubers are eaten, but are chiefly used for 'seed.' These species, however, he identifies with *D. alata*.

In the Stove at the Cambridge Botanic Garden there is a plant of *D. sativa* which produces in the axils of the leaves of its annual shoots, large rounded tubers, six or more inches in diameter and weighing as much as a pound or a pound and a half each. When mature these tubers are greyish or brown in colour, and present depressions in the surface which bear resemblances to those containing the 'eyes' in a potato. In addition to the tuber, the leaf-axil bears several (in some cases as many as eight) long, and very slender, pendulous spikes of flowers. It may be noted that these flowers are structurally hermaphrodite, although those in the wild forms of the plant are unisexual. Wight⁵ also notes that when cultivated, the flowers tend to become bisexual.

In order to observe the mode of origin and structure of the axillary tubers, successive series of sections were cut with a microtome. The plane of the sections is that which is

¹ Hooker, *Flora of British India*, vol. vi, p. 295 ('94).

² Bentham, *Flora Australiensis*, vol. vi, p. 461 ('73).

³ Bentham, *Flora Hongkong.*, p. 368 ('61).

⁴ Trimmen and Hooker, *Flora of Ceylon*, vol. iv, p. 278 ('98).

⁵ Wight, *Icones*, vol. iii; Description of Plate 878.

common to the stem and petiole, as far as this is possible on account of the difficulties which occur owing to torsion. Hence the sections are chiefly longitudinal. It will be convenient to trace the growth of the tubers by describing these sections from the apex of the stem downwards.

A median longitudinal section through the apex of a shoot, shows at each node a young leaf arching over the buds in its axil. Most of these buds, which generally number as many as six or eight, are the young spikes of flowers. At the young nodes the buds are undifferentiated and placed on a more or less conical mass of tissue in the leaf-axil.

Four or five nodes from the apex the differentiation of the buds has proceeded so rapidly that the two or three nearest to the stem have attained to the condition of elongated peduncles bearing lateral flower-buds. The youngest buds, nearest to the subtending leaf, are still rudimentary. The peduncles are arranged in pairs in the leaf-axils, the older anterior and the younger posterior. The youngest buds, posterior to the peduncles, are solitary. At this stage there is no trace of a tuber in the leaf-axil.

Somewhat lower down the stem the first beginning of the tuber is seen as a slight swelling below the youngest bud (Pl. XXVI, Fig. 1).

Sections through a node in which the young tuber is visible to the naked eye show that it is at this stage already distinctly separated from the surrounding tissues. Between the youngest of the peduncles and the tuber, in the series of sections examined, there was a single median vegetative bud, much less developed than those which form the peduncles. The tuber itself had two rudimentary buds lying in the median plane, one near the point of attachment of the tuber and the other more remote, and posterior. These buds caused angular projections on the tuber. The tissue lying a little below the cortex, between the buds, and especially between the posterior bud and the attachment of the tuber, was meristematic, and the most rapidly growing part of the structure.

In a young stem in another plant, grown under peculiar

circumstances to be described later¹, the arrangement of the structures in the leaf-axils was as follows. Between the main stem and the subtending leaf, and nearest to the stem, was one median axillary branch, immediately below and in front of this was the tuber on which were three buds. One, which was most developed, was just at the point of attachment of the tuber. The other two were also close to the point of attachment, one on the anterior side and the other on the posterior.

Two well-developed roots were formed on some tubers, one on each side of the biggest bud of the tuber (Fig. 5).

These buds, and the axillary branch, appear to be homologous with the impaired median buds in those leaf-axils which bear peduncles as well as tubers, but when flowering branches are produced the median vegetative branch remains undeveloped.

In a quite small tuber, about two mm. in diameter, the tissues are becoming differentiated. Within the epidermis is a cortex of parenchymatous cells containing chlorophyll. Many of these cells are enlarged and contain raphides. Beneath the cortex is a meristematic zone, which is most marked on the posterior side of the tuber. The central part consists of parenchymatous cells which already contain a considerable quantity of starch. Some enlarged cells are filled with raphides.

The two smaller buds are still, and remain for an indefinite time, quite rudimentary. They are in some cases entirely, in other cases partially, enclosed by the scale-like covering (Fig. 2).

A tuber about the size of a pea is covered with a single layer of epidermal cells beneath which is a thick layer, about twelve or fifteen cells deep, of cortical parenchyma which is brown in colour and beginning to lose its cell-contents. Then follows a zone of meristematic cells, separating the cortex from the central part which is made up of parenchymatous

¹ See p. 497.

cells, containing no starch. Cells with raphides occur in both cortex and medulla. At this stage there is no cork.

A tuber about three inches in diameter shows in addition to the buds large numbers of circular areas indicating the position of adventitious roots. The roots are produced most abundantly on the side of the tuber nearest to the point of attachment, although this is the side which also produces buds, and which, moreover, is turned towards the light as the tuber hangs on the stem. It should be stated that in the greenhouse the stem is trained horizontally on wires immediately under the glass roof. The question arises, does the stem grow vertically when wild, so that the roots will then be formed on the shaded side?

In a tuber of this size the structure is the same as in younger stages except that a layer of cork has been developed externally to the cortex, and replacing the epidermis. It arises in the cells immediately below the epidermis. Young vascular bundles, and also the adventitious roots, are formed from the meristematic zone.

When a large tuber is planted, one of the buds begins to grow rapidly and forms a strong shoot, from the base of which a large number of adventitious roots are produced (Figs. 3 and 6). No use is made of the numerous well-developed roots already formed in the tuber. Should the first formed shoot meet with an accident, as happened in the case of one of the tubers planted in the Botanic Garden, another bud develops. The formation of axillary tubers on these shoots begins very early.

The shoot arising from one of the tubers planted at the Botanic Garden was kept comparatively short, about a dozen or more nodes only being preserved. It was pegged down to the earth at intervals in order to see if it would form underground tubers at these points, as this is said to be a method of cultivation used by the Chinese to produce large crops of small tubers resembling potatoes¹. At these

¹ Economic Products of India, p. 123 ('90).

nodes tubers were not formed, though they were developed at all the free nodes.

When the plant died down in the autumn a new underground tuber had been formed at the base of the annual stem, of smaller size than the original axillary one, which had been emptied of its contents but still remained attached to the stem (Fig. 6). The new tuber was covered with roots, which were arranged in more or less definite rings on its surface, as is the case also in the undeveloped roots of the axillary tubers (cf. Figs. 3 and 6).

The axillary tubers of another species of *Dioscorea*, perhaps *D. divaricata*, behaved differently when planted. This species grows out of doors at the Botanic Garden, and in the autumn it produces numerous small tubers which are about the size of peas, either rather smaller or a little larger. Usually there is only one in the axil of each leaf, but there may be two or even three. As in *D. sativa*, they possess both buds and roots. In both species the structure is similar. When one of these little tubers is planted it sends up one shoot, and begins itself (Fig. 7) to grow downwards as an elongated mass, while at the same time it forms, like *D. sativa*, a comparatively large new tuber, often of irregular shape.

Some interesting points were noted in tubers which formed shoots in the laboratory, without water, but in the presence of light. In two cases a single stem, six or eight feet long, was produced by each tuber, while from two other points smaller shoots were formed. It is noteworthy that at the bases of these stems buds were formed, which must be adventitious. Although the stems were so long and were also comparatively thick, the leaves were scarcely developed at all, and the axillary branches remained small (Figs. 3 and 4). Nevertheless, small tubers about half an inch in diameter were formed in the axils of most of these arrested leaves. The number and arrangement of the buds on these tubers and in the leaf-axils which bore them have been described above (p. 495). Round the bases of all the shoots numerous adventitious roots were formed, some of which became about

half an inch long and about an eighth wide. They were covered by a rough, almost scaly epidermis.

It is evident from the foregoing observations that the axillary tubers of *Dioscorea sativa* are of the morphological nature of stems bearing buds which are both axillary and adventitious, and roots which are adventitious. Apparently the underground tubers in this species are of the same morphological value. When developed below the ground, the roots in the tuber grow out and become functional, whereas in the aërial tuber, even if this be planted, they never appear outside the epidermis or periderm. This may be due to the fact that in large tubers the external tissues are thick and corky, so that the root may not be able to penetrate them.

Concerning the morphological value of the different kinds of tubers in various species of *Dioscorea* there is diversity of opinion.

De Bary¹ places the underground tubers in three categories:—

- (1) Tuberous swollen roots, e. g. *Dioscorea Batatas*.
- (2) Rhizomes with scaly leaves and composed of many internodes, e. g. *Dioscorea villosa*.
- (3) Leafless tubers, resulting from the swelling of the first epicotyledonary internode of the seedling, e. g. *Tamus communis*, *T. polycarpus*, *Testudinaria*, and many species of *Dioscorea*.

There seems to be a general consensus of opinion that the underground tubers of *D. Batatas* are true roots². According to Royer³ the perennial part of *D. Batatas* is a small almost globular body about the size of a hazel nut, situated at the top of, but distinct from, the tuberous root. Each year this perennial organ, which is morphologically a stem, produces a twining stem and a tuberous root, and it is itself marked

¹ De Bary, Comp. Anat. of the Phanerogams and Ferns, Eng. edit., p. 622 ('84).

² De Bary, l. c. Engel and Prantl, vol. ii, 5, p. 131 ('88).

³ Royer, Le tubercle de l'Ignome est une racine, mais non pas un rhizome. Bull. de la Soc. Bot. de France, vol. xxx, p. 225 ('83).

with two series of cicatrices which result from the annual detachment of the stem and root respectively.

Bucherer¹ points out that the tuber of *D. Batatas* has the structure of a root.

Only one author, Morot², appears to hold that these underground tubers are stems. He regards each of them as consisting of a single reduced internode. In this way he explains the absence of scales.

The majority of tubers in the Dioscoreaceae are, however, undoubtedly stem-structures. They are considered to be rhizomes in *D. bulbifera* and *D. pentaphylla*³ and, as already mentioned, in *D. villosa*. In *D. aculeata*³ short stolons bear, at their distal ends, rounded tubers about the size of the fist. One plant bears as many as seven or eight⁴.

The tuber is in some species the first internode of the stem, and is therefore leafless, e. g. *Tamus*, *Testudinaria*, and some species of *Dioscorea*. According to von Mohl⁵, the tuber of *Tamus* (*Testudinaria*) *Elephantipes* is to be regarded as an adventitious bud, which each year is formed anew between the wood and the cortex of the tuber-like stem. Consequently the annual shoots are developed from adventitious buds.

The axillary tubers are, in all cases, stem-structures, the only question being as to the number and nature of the buds which occur upon them. Such aërial tubers are formed on many species; their development has been described in *D. Batatas*, Decsne, and *Helmia bulbifera*, Kunth, by Queva⁶. In *D. Batatas* the tuber arises as a single axillary bud. There are in each axil also one or two other buds which are branches. The surface of the tuber is covered with slight

¹ Bucherer, Beiträge zur Morphologie und Anatomie der Dioscoreaceen. Bot. Centr., vol. xliii, p. 121 ('90).

² Morot; see Royer, l. c., p. 227.

³ Vieillard, Plantes utiles de la Nouvelle Calédonie. Ann. des Sci. Nat., 4^e sér., vol. xvi, pp. 39-40 ('62).

⁴ Sagot, Des Ignames. Bull. de la Soc. Bot. de France, vol. xviii, p. 309 ('71).

⁵ Von Mohl, Untersuchungen über den Mittelstock von *Tamus Elephantipes*, L. Verm. Schrift. Bot. Inhalts, p. 193 ('36).

⁶ Queva, Les Bulbilles des Dioscorées. Comptes Rendus des Séances de l'Acad. des Sciences, vol. cxvii, pp. 316-318 ('93).

elevations which mark the position of adventitious roots. The plant described by Queva as *Helmia bulbifera*, Kunth, appears to be the one which was considered above to be identical with *Dioscorea sativa*, Linn., i. e. the species under consideration in this paper. This opinion is confirmed by a comparison of the development of the tubers as described by Queva and by the present writer. The descriptions may be briefly compared in some points. Queva observes that the growing points of the original buds (usually three) remain in the plane of symmetry of the organ, which corresponds with that of the leaf. On the mature tuber the growing point of the posterior bud is placed on the lower surface, that of the middle bud on the upper side, while the anterior bud remains near the point of attachment of the tuber. The observations made on the tubers grown at Cambridge show that in large tubers the three buds lose their original positions and all come to lie close to the point of attachment. They are therefore all on one side of the tuber in an advantageous position when they begin to grow. Adventitious buds are also formed, close to the original buds.

The abundant formation of axillary tubers in many species of *Dioscorea* seems as if it were connected with the fact that these plants do not appear to form seed readily. The experiments with the plants grown without water in the laboratory show that the tubers, and the shoots which they produce, have a great power of resisting drought, which would often kill the more delicate tissues of a seedling. Vegetative reproduction by means of axillary tubers appears to a large extent to have superseded sexual reproduction in this genus.

In conclusion I wish to thank Professor Marshall Ward for allowing me to work in the University Botanical Laboratory, and for the help which he always so willingly gives.

EXPLANATION OF FIGURES IN PLATE XXVI.

Illustrating Miss Dale's paper on *Dioscorea sativa*.

Fig. 1. Longitudinal section through a node of *Dioscorea sativa*, showing young tuber and young inflorescences. *s.* main stem; *a.* petiole; *b.* young inflorescences; *c.* bud on young tuber.

Fig. 2. Longitudinal section through older tuber about 2 mm. in diameter. *s.* main stem; *b.* young inflorescence; *c.* buds on tuber.

Fig. 3. Old axillary tuber which has produced long shoots without being planted or watered. *s.* stems; *r.*¹ undeveloped adventitious roots on the tuber; *r.*² young adventitious roots on the young shoots; *sc.* scales; *l.* arrested leaves with branches in their axils.

Fig. 4. Part of one of the shoots produced by an unplanted tuber. *t.* tuber; *l.* arrested leaf; *b.* bud; *br.* branch.

Fig. 5. A tuber on a similar branch. *t.* tuber; *a.* petiole; *b.* branch; *c*¹, *c*², *c*³, buds; *d.* root.

Fig. 6. Axillary tuber *T*, which has been planted and produced a new tuber, *t*; *s* 1, scar of first developed bud, injured by an accident; *s* 2, second stem replacing the injured one; *r.*¹ undeveloped adventitious roots on old tuber; *r.*² adventitious roots on young tuber.

Fig. 7. Behaviour of the axillary tuber *T*, of another species of *Dioscorea*, perhaps *D. divaricata*. *T*¹, newly developed part of original tuber; *t.* new tuber; *b.* bud on original tuber.



Fig. 1.



Fig.

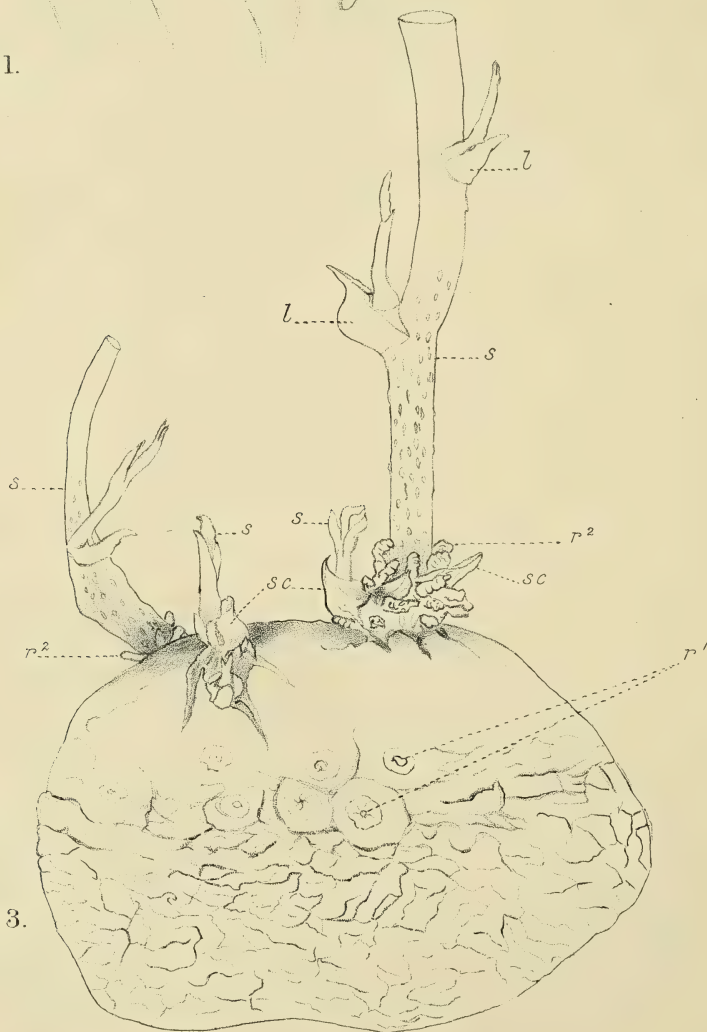


Fig. 3.



Fig. 4.

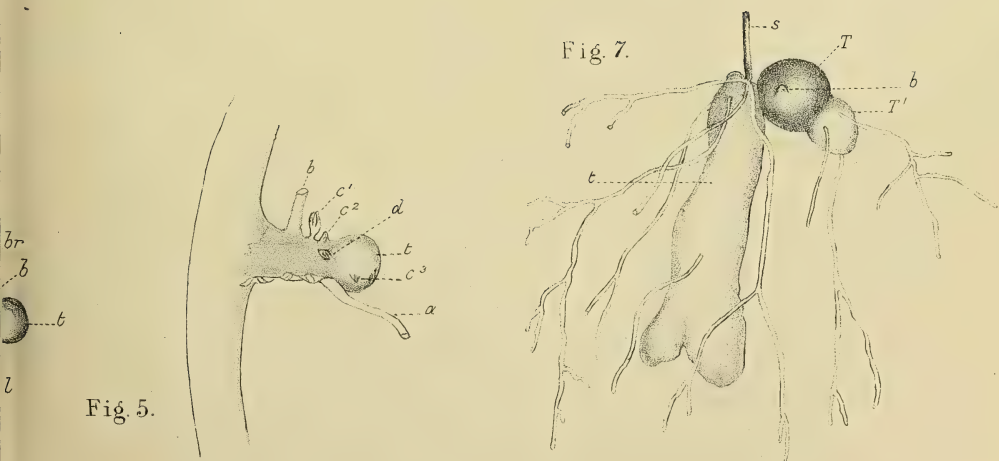
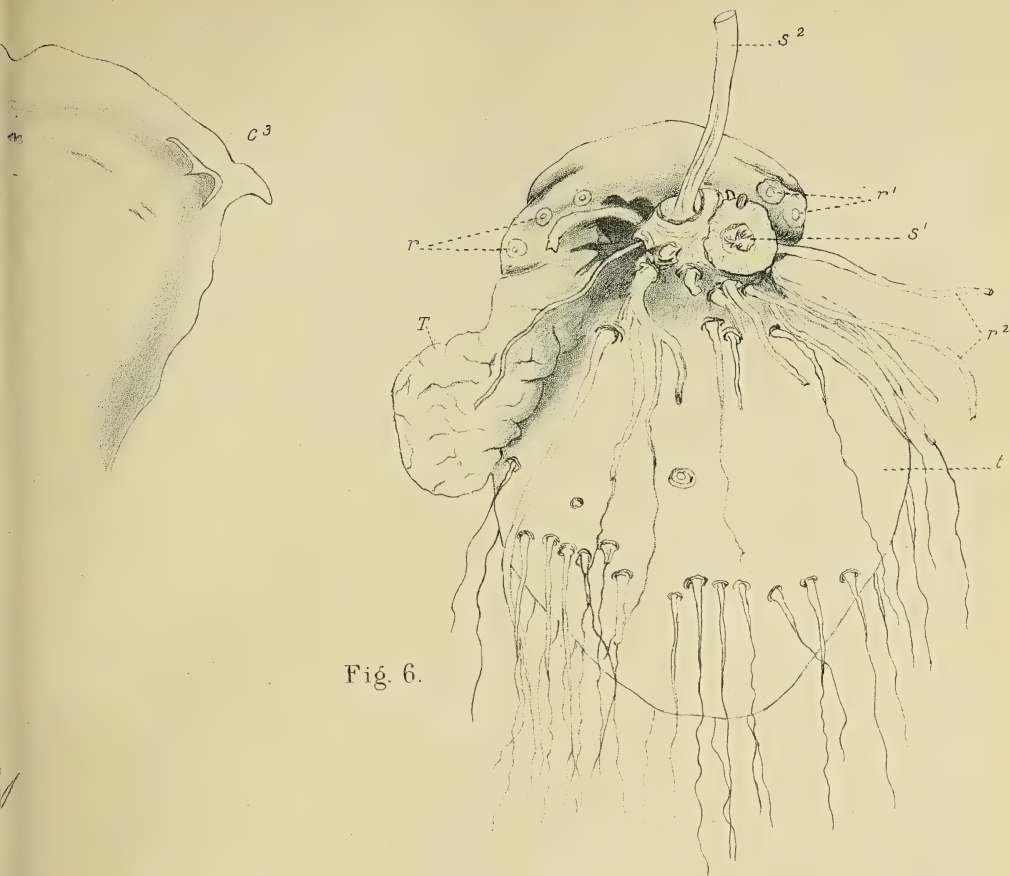




Fig. 1.



Fig. 2.

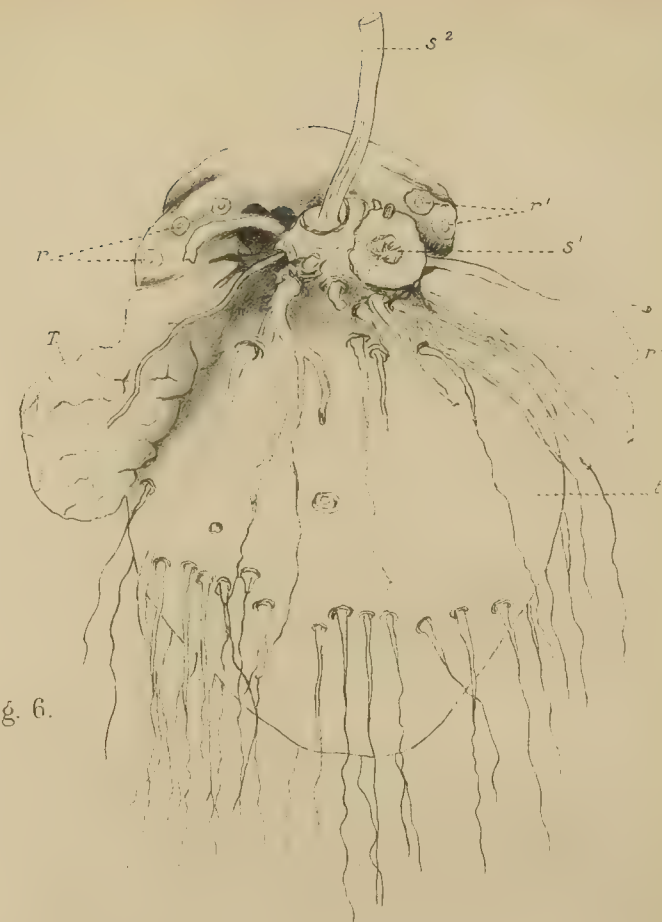


Fig. 6.

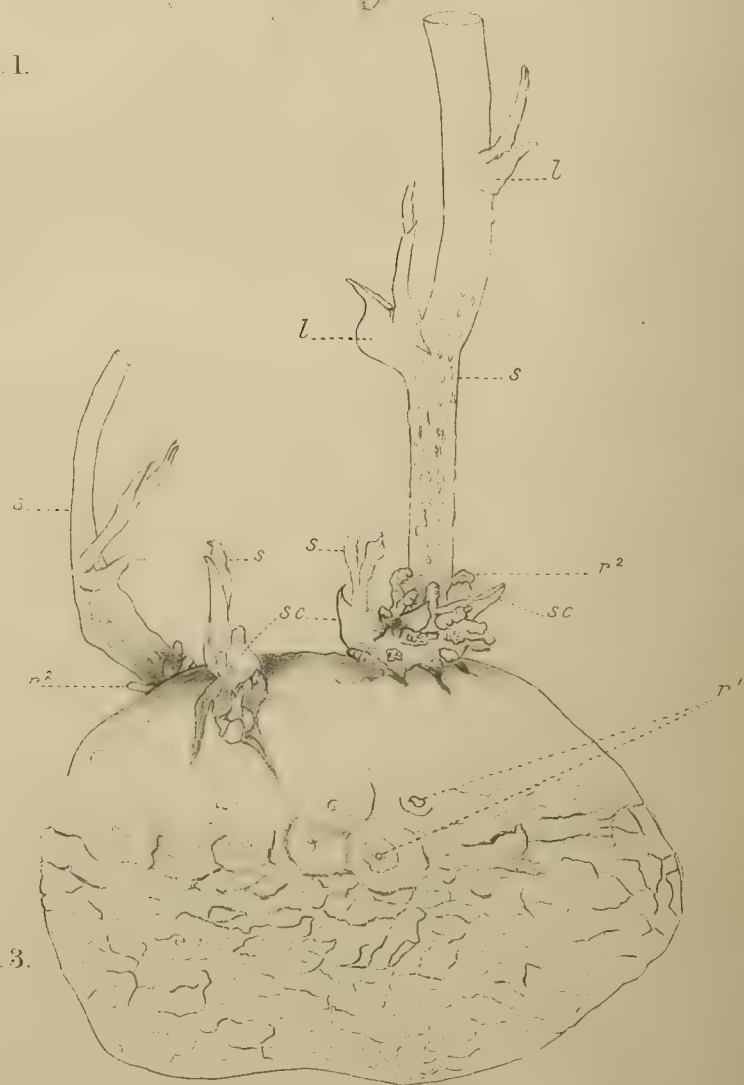


Fig. 3.



Fig. 4.

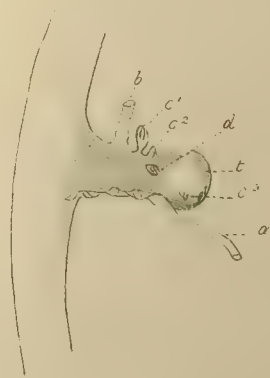


Fig. 5.



Fig. 7.

On Apospory in *Anthoceros laevis*.

BY

WILLIAM H. LANG, M.B., D.Sc.,

Lecturer in Botany at Queen Margaret College, Glasgow University.

—+—
With Plate XXVII.
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THE fact that under certain conditions the vegetative cells of the asexual generation could produce the sexual generation, and that the origin of the latter was not necessarily connected with the spore, was discovered in the Mosses. Pringsheim¹ first described it for three species (*Hypnum cupressiforme*, *H. serpens*, and *Bryum caespitosum*), while the independent work of Stahl² was done on *Ceratodon purpureus*. From Pringsheim's account and figures it appears that the seta of the ripe capsule was cut into short pieces, which were laid on damp sand; in Stahl's experiments the sporogonia were either simply pulled away from the moss-plant or cut off above their point of attachment to the latter. In both series of experiments, whether the sporogonia were more or less cut up or were uninjured, protonemal filaments ultimately arose from certain of their cells. Brizi³ has since shown that in *Funaria hygrometrica* this may take place from capsules still attached to the moss-plant. Without referring in detail

¹ Monatsb. d. k. Akad. Wiss., Berlin, 1876. Jahrb. wiss. Bot., Bd. xi, 1877.

² Bot. Zeit., 1876, p. 689.

³ Ann. d. R. Inst. Bot. Rom., vol. v., p. 54, 1893.

[Annals of Botany, Vol. XV. No. LIX. September, 1901.]

to the corresponding phenomenon since discovered in Leptosporangiate Ferns, it may be pointed out that in these plants also the sexual generation may arise from an uninjured sporophyte, or its development may require to be induced by laying pieces of a leaf on damp soil.

Although discovered in Mosses and subsequently found in a number of Ferns, apospory is not, so far as I know, hitherto recorded for any Liverwort. While working in the laboratory of the Peradeniya Botanic Gardens it occurred to me that similar treatment of the sporogonia of *Anthoceros laevis*, L., which grows abundantly in the gardens, might induce apospory. Accordingly a number of young, unopened sporogonia were taken, and the portion projecting from the calyptra cut up into lengths of about 5 mm. The pieces were laid on damp sand and covered with a bell-glass. By the end of a month they were more or less decayed, and of a yellowish-green tint. From the cut ends and sometimes from the surface, however, small outgrowths of a deep green colour could be seen to have arisen (Pl. XXVII, Fig. 1). The culture underwent but little change during the next fortnight, when it had to be stopped owing to my leaving Peradeniya for some months. None of the new growths had assumed the flattened form of the thallus, but their resemblance to the latter in its early stages and the origin of rhizoids from them (Fig. 2) sufficiently demonstrated that it was a case of apospory. Further, both in the position in which the new growths appear and in the method by which their development was induced, this case of apospory in a Liverwort corresponds closely with those recorded for the Mosses.

Before describing the pieces of sporogonium from this culture, some points in the normal structure of the corresponding region must be briefly referred to. As in most species of *Anthoceros*, the epidermis consists of long narrow cells; within this the wall of the sporogonium consists of about five layers of oblong parenchymatous cells, about four times as long as broad; the spores with the intervening trabeculae surround the columella, which is composed of

long narrow cells. In the epidermal cells are small starch-grains; in the other cells of the wall a considerable amount of starch may be present within the single large chloroplast, while the cells of the columella and trabeculae contain little or no starch. The young spores contain abundant starch. Thus the cells of the five inner layers of the wall are the least specialized of the sterile cells of the sporogonium and contain the greatest amount of reserve material, while the presence of a healthy chloroplast in them enables this supply to be increased.

After cultivation on damp sand the pieces of sporogonium became more or less disintegrated, and many of the cells composing them were evidently dead or dying. Others, however, were still healthy. Such cells occur throughout the wall, most commonly in the layers close to the epidermis, and they stand out prominently when stained with iodine owing to their chloroplasts containing starch. Many of these living cells had not divided, and only differed from the corresponding cells in the uninjured sporogonium in having rounded themselves off somewhat from their neighbours. In others, however, cell-divisions had taken place. These divisions may commence in cells which are still covered by the epidermis (Figs. 4, 5), but the new growths develop more rapidly when they are on a free surface of the piece of sporogonium. Thus those growths which arise from the cut surfaces at the ends (Fig. 1) develop first and, as the figure shows, may attain a considerable size while the epidermis is still intact. As the disintegration proceeds, however, the epidermis gets broken and in places stripped off, and then growths arising from the cells beneath, such as that shown in Fig. 3, are enabled to continue their development.

In almost every case each new growth appeared to owe its origin to a single cell of the sporogonium (cf. Figs. 3-6). The divisions, which take place in the cell, are subject to considerable variation, but the first is usually transverse and often separates a lower cell, in which few or no further divisions occur, from an upper, which gives rise to the new

growth (Fig. 3). In other cases this contrast is wanting. The general sequence of the early divisions in the cells giving rise to the new growths, and the differences between individual cases, are closely parallel to the early stages of germination of the spore of *Anthoceros*. The oldest buds obtained also resemble a stage in the germination of the spore, as will be seen by comparing Fig. 2 with such a figure as that on p. 138 of Campbell's 'Mosses and Ferns,' and that they are gametophytic is further indicated by the origin of rhizoids from them. As mentioned above the culture had to be stopped at this stage and therefore the comparison cannot at present be carried further.

There remains for consideration the evidence that these new growths, which have been seen to be gametophytic, are truly aposporous. Three possible sources of the buds had to be considered; they might have arisen from spores present in the sporogonium or accidentally introduced into the culture, or from the undivided spore-mother-cells in the spore-sac, or finally from the sterile tissue of the sporogonium. Even from external examination of the pieces of sporogonium, removed from the culture when fresh, the third of these alternatives seemed the probable one, for, when the growths arose from the cut end, they sprang from close beneath the surface, while, if from the side of the piece, the whole growth corresponded in position and size to one of the cells of the wall and was attached closely to the neighbouring cells. No trace of a ruptured spore-membrane was ever visible. The actual proof, however, could only be obtained by the study of sections; these showed that the growths might start beneath the unbroken epidermis from cells of the wall surrounded on all sides by their neighbours. Most commonly they arise from subepidermal cells, but they may start in any of the layers of the wall down to that which bounds the sporogenous layer (Figs. 4, 5). The same holds for growths originating from the cut ends (Fig. 6).

The positive evidence is thus sufficient to establish the fact of apospory in this Liverwort, and even did cells of the sporo-

genous tissue or the young spores undergo development, it would merely increase the difficulty of demonstration in the case of any particular growth. In the majority of the pieces of young sporogonia experimented with, the young spores simply disintegrated without any attempt at further development. In one case, however, growth had taken place within the spore-sac leading to the presence of young plants consisting of a few cells; from this single example it was impossible to decide with certainty whether these were referable to young spores or to spore-mother-cells.

The small number of specimens obtained from this culture did not afford material for the study of the cytological changes, which may occur in the cells destined to give rise to a gametophyte. The frequency with which the new growths start from single sterile cells of the sporogonium might make this and similar cases of apospory suitable for such observations. Even in the absence of observations on the behaviour of the nucleus, certain considerations on the point of view, from which the phenomenon of apospory is best regarded, are suggested by this case, and may be briefly referred to without entering on any general discussion of apospory.

In the normal life-history of such a plant as *Anthoceros* the fact that the fertilized ovum gives rise to the sporophyte and the spore to the gametophyte must be regarded from two points of view. In the origin of both generations the start is made from a single cell (zygote, spore) and these cells in the two cases have had very different histories. The zygote has arisen by the fusion of two cells, while the formation of the spore is preceded by the reduction of chromosomes in the spore-mother-cell. But it is not sufficient in considering the remarkable fact—that these two sorts of reproductive cells in the same species give rise to distinct and very different stages in the life-history—to note their different origin. The conditions under which they undergo their further development are also very different in the two cases. The spore, separated from the parent plant, falls on the ground, and under suitable conditions of moisture, warmth, and illumination develops

into the thallus, the material for the growth of which is provided by its chlorophyll-containing cells. The zygote enclosed in the venter of the archegonium is at first wholly dependent on the gametophyte, and the sporogonium developed from it never becomes free from the latter.

Similarly, with regard to the explanation of such a deviation from the ordinary life-cycle as apospory, both the character of the cells, from which the development proceeds, and the nature of the conditions, to which they are exposed during development, require consideration. As regards the former point, it has been shown above that certain cells or groups of cells of the sporogonial wall remain alive while others die and disintegrate. The living cells thus become to some degree isolated in position and, as may fairly be assumed, physiologically also. The cells of the cut ends, which were seen to commence growth earlier, are partially isolated by the section through the sporogonium. These cells of the wall have also been seen to be the least specialized in the sporogonium, and they are capable of the continued manufacture of organic material. Whatever the changes involved in the reconstitution of these cells may be, all these facts point to there being a general physiological distinction between them and the other tissues of the sporogonium. In *Anthoceros*, as in the cases of apospory among Ferns, the sporogenous tissue itself, if it is distinguishable, does not take part in the origin of the new growths. The only apparent exception to this, as a general rule, is in the case of the mosses, in which Pringsheim points out that the zone of the seta, from which protonemal filaments arise, corresponds to that zone in the capsule between columella and wall in which the archesporium lies. But the whole of the seta is sterile tissue and there is no evidence that archesporial cells themselves give rise to new growths. On the other hand, Stahl's observation, that apospory could be induced in the cells of the capsule-wall, shows clearly that there is no necessary connexion in the Mosses, between the archesporial region and apospory. In Mosses, as in *Anthoceros*, the growth starts from little-specialized

parenchymatous cells, which have fairly thin walls and contain chlorophyll, and probably these characters are of much greater importance than the position of the cells in relation to the archesporium.

The conditions, to which these cells were exposed, must in the second place be considered. They were essentially similar to those under which the spores develop into the thallus (moisture, sufficient temperature, light), while like the spores these cells were more or less isolated from their neighbours. Under these circumstances they developed into the gametophyte. The most important factor in the environment of the young sporophyte, its position in and nourishment by the gametophyte, is entirely absent, and it is hardly too much to say that it would be surprising if under these circumstances the cells of a decaying sporophyte of a Liverwort or Moss produced sporogonia. The cases of apospory in Bryophytes appear to indicate the important influence of the environment in determining the origin of a gametophyte from an isolated cell of the sporogonium. All the sporogonia which have been experimented with were normal, and were obtained from wild plants; there is here no question of a predisposition towards apospory such as complicates the problem in the case of some Ferns. The experimental conditions to which the cells have been submitted have brought into view certain of their properties which are not called into play in the normal life-history.

The observations made on this case of apospory only serve to suggest the use of a thorough study of the conditions, to which the tissues experimented with are exposed, both as regards the inorganic environment and the alterations in their relations to neighbouring tissues. A knowledge of the facts regarding the influence of the change of environment, as well as those relating to the possible reconstitution of those cells of the sporophyte, which develop into the other generation, is the necessary preliminary to any estimate of the evidence afforded by apospory as to the nature of alternation of generations. Util further knowledge is obtained on these points it appears

equally unsafe to assume that this deviation from the normal life-history is a reversion, or to dismiss it as a mere sport with no phylogenetic bearing.

In conclusion I have to express my indebtedness to the Director of the Royal Botanic Gardens at Peradeniya for the use of a table in the Laboratory where the culture was made and the observations commenced.

EXPLANATION OF FIGURES IN PLATE XXVII.

Illustrating Dr. Lang's paper on Apospory in *Anthoceros*.

Fig. 1. End of a piece of sporogonium after six weeks' cultivation, showing the aposporously-produced growths arising from the cut surface. $\times 80$.

Fig. 2. Surface-view of a growth like those in Fig. 1, which has produced a rhizoid. $\times 375$.

Fig. 3. Surface-view of a growth referable to a single cell of the sporogonial wall, exposed by the separation of the epidermis. $\times 375$.

Fig. 4. Longitudinal section showing the rounding off and division of a number of cells of the partially disintegrated wall. $\times 200$.

Fig. 5. Similar section to Fig. 4. The wall is hardly disintegrated; the two new growths are covered by epidermis. $\times 200$.

Fig. 6. Section through the end of a piece of sporogonium, showing the origin of two growths from different layers of the wall. $\times 200$.

Fig. 7. Section through one of the most advanced growths like that in Fig. 2. $\times 200$.



Fig. 1.



Fig. 2.

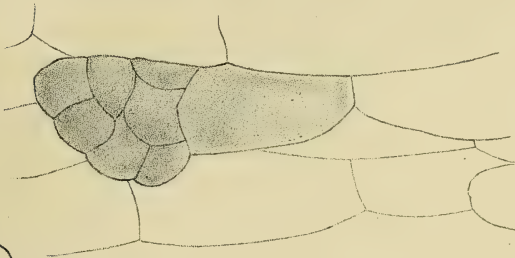


Fig. 3.

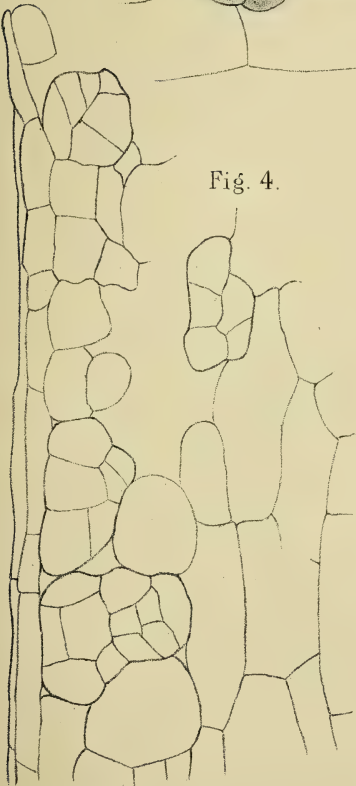


Fig. 4.

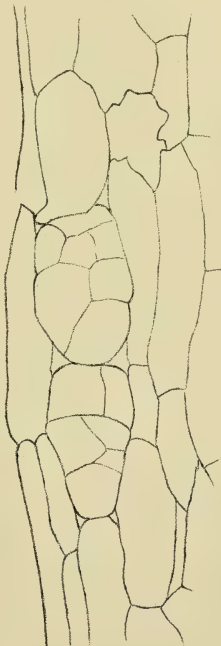


Fig. 5.

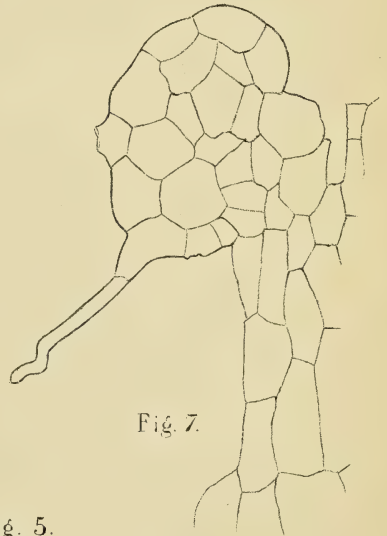


Fig. 6.



Fig. 7.

On the Economic Importance of 'Nitragin.'

BY

MARIA DAWSON, D.Sc. (Lond. and Wales),

Late 1851 Exhibition Science Research Scholar.

IN connexion with the comparatively recent rise of Agriculture as a practical science, there is perhaps no more important question than the supply of an adequate quantity of suitable nitrogenous food to plants, from which has arisen the study of the part played by the innumerable micro-organisms of the soil and air in the regulation of this supply, both quantitatively and qualitatively.

The special interest of the Leguminosae in this connexion has been recognized ever since the classical researches of Boussingault, and from that time there has been an unbroken series of investigations—in particular those of Lawes, Gilbert, and Pugh in England and Hellriegel and Wilfarth in Germany—upon the phenomena involved in the increased nitrogen content of the soil, found to be a constant result of the cultivation of a Leguminous crop. The first important stage towards the explanation of these phenomena was reached by the determination that this increase in nitrogen was directly correlated with the presence upon the roots of the Leguminous plants of nodules, which owed their formation to the action of parasitic micro-organisms present in the soil. Beyerinck's discovery that the organisms were capable of growth on nutrient media, outside the plant, and that they could be obtained direct from the culture-soils, led to very

many experiments upon plants in soils inoculated with artificial cultures of the organisms or with decoctions of soils in which the plant in question had been already cultivated. Such work as this led to the introduction by Nobbe and Hiltner of the substance 'Nitragin,' i.e. pure cultures of the nodule-organisms derived from different Leguminous plants, on a commercial scale for use in practical agriculture.

Although so much work has been done upon the relation existing between the presence or absence of Leguminous nodules and the proportion of nitrogenous material found in the culture-soils, but few workers have used the pure culture 'Nitragin' in exact experiments of this character, and so far as I am aware, in all cases, in which this material has been used, the conditions of the experiment have been very uncertain, owing chiefly to non-sterilized soils only being employed as culture media. Though not wishing to undervalue such experiments, I may point out that with such very complex conditions, it is impossible to fix upon any one variable condition as the certain cause of any observed effect produced in the crops, and indeed recent work upon this subject has tended to prove more and more clearly that not only is this problem of modern agriculture one of special difficulty, but that we can only hope to come to an adequate explanation of the facts involved by bringing to bear upon them evidence derived from the study of many apparently side issues.

Since 'Nitragin' was intended for use on a commercial scale and on any kind of soil, it seemed advisable that careful test experiments should be undertaken in a large number of different districts, and it was in order to furnish one such set of experiments that the work, to be briefly described in this paper, was undertaken during the summers of 1898-1900 in the Cambridge University Botanical Laboratory and Gardens.

Throughout the work I had the privilege of the invaluable help and advice of Professor Marshall Ward, and I wish to take this opportunity of acknowledging my indebtedness to him. The results of some of this work have already been

referred to in my earlier papers¹ on the subject of the nodules of Leguminous plants, but further details were reserved in order to secure the advantage of a comparison of the experiments of three consecutive years, before any definite opinion was offered upon so complicated a subject.

The experiments referred to in this paper were of two distinct types:—

1. The plants were grown in media previously sterilized by heat [approximately 200° C. for twenty-four hours], and throughout the experiment every precaution was taken to prevent any chance infection of the roots.

2. The plants were grown in the open air on unsterilized media.

In every case the same species was chosen for investigation, viz. *Pisum sativum*.

In the experiments on *sterilized media* the plants were grown in large pots containing respectively ordinary garden soil, a gravelly subsoil, pure silver-sand devoid of nitrogenous compounds, and a similar sand supplied with potassium nitrate. In each case one half of the pots were sown with seeds, previously inoculated with 'Nitragin,' whilst the other half contained control plants without inoculation. Before sowing, the seeds themselves were sterilized by immersion in a one per cent. solution of mercuric chloride for fifteen minutes².

Experiments of this kind were carried out in three consecutive years, and in each year the crops were allowed to grow for about three months, that is until the pods were ripened, and throughout the time were watered with boiled distilled water only. At the end of this time the plants were carefully removed from the soil, and the roots thoroughly washed. A record was then made of the number of plants bearing nodules, the number of nodules formed and the dry

¹ Phil. Trans. Roy. Soc., 1899 and 1900.

² The use of this re-agent for cleansing the seeds was justified by the results of a preliminary experiment in which I found that 15 minutes' treatment with a one per cent. solution of mercuric chloride was without injurious effect on pea seeds, though a longer application killed the embryo.

weight of the crops obtained. During the course of these experiments over 800 plants have been under investigation in this manner.

With regard to the relative weights of the crops the results showed that on ordinary garden soil, on sand, and on sand manured with nitrate, inoculation with 'Nitragin' is accompanied by a loss of weight in the crop, but that a small increase is secured on gravelly subsoil.

In sand-grown plants a considerable increase in the crop was produced by a supply of nitrates alone, but inoculation with 'Nitragin' in the presence of a sufficient supply of nitrogenous food, whether in the form of humus or of potassium nitrate, is not beneficial.

This set of experiments also showed clearly the extreme difficulty of securing sterile conditions in the culture-soils for a considerable period of time, and emphasized the fact of the undoubted ubiquity of the nodule-organisms in air and soils alike; for, in spite of the greatest care in all manipulations to prevent chance infection of the roots, nodules were frequently found on the roots of control-plants. It is scarcely possible that this was due to incomplete sterilization of the soils, for I have repeatedly grown peas and other Leguminous plants on media, treated in a similar manner for a period not exceeding six weeks, without any trace of infection. In addition, it should be pointed out that the nodules, when present on control-plants, were always fewer in number, smaller and more crowded together than those borne by plants inoculated with 'Nitragin.' Moreover the frequent occurrence of the fructification of *Peziza confluens* on these sterilized media shows how easily air-borne germs could be introduced. It would seem therefore that these nodule-organisms must be regarded as amongst the most ubiquitous of known organisms and, like those of putrefaction and fermentation, extremely difficult to remove with certainty for a considerable length of time. At one stage in the experiments it was observed that though no very appreciable difference could be detected between the vegetative organs

of the plants with and without inoculation, the pods borne by inoculated plants were ripening more quickly than those borne by the plants without inoculation.

This observation lends support to the view recently put forward by Mattiolo¹ that the root-nodules are organs for the elaboration of the albuminous materials required in the formation of the seeds. He found that if the formation of the fruit was prevented by the removal of all the young flower-buds, the contents of the nodules were not absorbed by the plants; whilst on the contrary the plants producing a normal quantity of fruit bore nodules which were completely emptied of their reserve stores of albuminous substances.

In the experiments on *unsterilized media* the plants were arranged—

1. In a parallel series of three sets of crops, grown in the summer of 1899, upon ordinary garden-soils and a gravelly subsoil with and without inoculation with 'Nitragin' and with and without an additional supply of nitrogenous food in the form of potassium nitrate.

2. In a series of crops grown in the summer of 1900, on ordinary garden-soil, clay, peat and loam, in order to test the effect of inoculation with 'Nitragin' on media of different chemical composition.

As before the plants were allowed to grow until the pods were ripened, and records were then made of the numbers of infections and the dry weight of the crops.

These open-air experiments, which involved the cultivation of nearly 700 plants, led—like those conducted on sterilized media—to a very unfavourable conclusion as regards the practical value of 'Nitragin.' The nodule-organisms were found to be present in all types of soil, though they seem to be less abundant in clay and peat, and in these particular soils alone a large increase in the number of infections resulted from inoculation with 'Nitragin.'

¹ Mattiolo, 'Sulla Influenza che la Estirpazione dei Fiori esercita sui Tubercoli radicali delle Pianta Leguminose,' 1900.

As regards the relative weights of the crops, an increase as the result of inoculation was observed on the gravelly subsoil only, and even here the increase was but small ; on peat, clay, loam or ordinary garden-soil, on the contrary, inoculation with 'Nitragin' proved to be both useless and superfluous.

In the report of the work undertaken during the year 1897, in the various experimental stations of Prussia¹, accounts are given of the use of 'Nitragin' with different genera of the Leguminosae and on soils of different characters. In general the results show that the artificially cultivated organisms are either quite useless for infection or superfluous. On some soils, e.g. sandy heaths and moorland, it was found that inoculation with soils known to contain the organisms in question was more beneficial to the crops than the use of the pure culture, 'Nitragin.'

Frank², in a discussion of these results, suggests that the failure of 'Nitragin' as an agricultural fertilizer of Leguminous crops may be due to a change of properties having been induced in the organisms by artificial cultivation, which change has lessened their virulence, and consequent action within the host-plants. He concludes from these facts that a more suitable culture-medium should be adopted in order to keep the properties of the organisms unchanged. It is to be noticed, however, that this author has not suggested of what this new medium should consist. Nobbe and Hiltner³ hold the view that this loss of virulence is due to the same nutrient gelatine being used in each case, whatever the organism cultivated, e.g. *Lupinus* organisms on pea-extract gelatine.

They consider that the special adaptation of the 'bacteria' to each host is dependent upon the presence of different food materials within each host. According to these authors the value of nodules to the Leguminous plant is in direct propor-

¹ Landwirthsch. Jahrb., 1898, Band XXVIII.

² Frank, 'Die bisher erzielten Ergebnisse der Nitraginimpfung.' Landwirthsch. Versuchsst., 1899.

³ Nobbe and Hiltner, 'Wie lässt sich die Wirkung des Nitragins erhöhen?' Landwirthsch. Versuchsst., 1899.

tion to the conversion of the 'bacteria' into bacteroids; unchanged 'bacteria' may be harmful rather than useful to the plant. They also state that if the plants are strengthened by the addition of small quantities of nitrogenous food to the soil, then nodules produced by artificially cultivated organisms are quite normal in their behaviour as nitrogen collectors. My own work has led me to an entirely opposite conclusion, viz.: that the more favourable the supply of nitrogenous food within the culture-media, the less likely is inoculation with 'Nitragin' to have any beneficial effect upon the crops.

In a general discussion of the kind of experiments referred to in this paper, it might justly be argued against the use of sterilized media that the conditions are here so altered that the results obtained cannot be regarded as comparable with those obtained under natural conditions.

On the other hand, the value of, and need for, experiments of this kind must be realized when we consider the unusual complexity of the conditions existing in any ordinary soil. In media, thoroughly sterilized by heat, these conditions are to a large extent under control, and the disturbing action of soil-Bacteria removed, though even here, as we have seen, the difficulty of guarding against infection by air-borne germs is immense. In ordinary soils in the open-air, the conditions regulating the supply of nourishment and in particular of nitrogenous food to the plants are in the highest degree complicated, for along with the action of the nodule-organisms is involved the action of the various nitrifying and denitrifying Bacteria at work within the soil, besides frequent variations in the environment due to changes in the atmospheric conditions.

In this connexion reference should be made to Richter's¹ investigations upon the effect of sterilization by heat, which causes considerable changes in the soil, in regard to its function as the main food-supply of plants—in particular, that the in-

¹ Richter, 'Über die Veränderungen welche der Boden durch das Sterilisieren erleidet.' Landwirthsch. Versuchszt., 1896.

soluble nitrogenous compounds are converted into more easily assimilated forms. Such a change in the nature of the food-supply would naturally lessen the plant's need of root-nodules as nitrogen-collectors. In ordinary soils, on the other hand, the processes of nitrification and denitrification, so continuously going on, produce frequent variations in the quantity and quality of the nitrogenous food available to higher plants; so that in the struggle for existence it may well be a distinct advantage to the Leguminosae to have the power to draw upon additional supplies, provided through the agency of the root-nodules.

Further, the action of the host in the probable absorption from the nodules of the products of the activity of the symbiotic or parasitic organisms, doubtless in its turn aids in securing the continuance of their action upon the nitrogenous constituents of the soil or air. For it is quite conceivable that an accumulation of the products of their own metabolism within the nodules would in time result in the death of the organisms, or at least would entirely inhibit the exercise of their special metabolic functions. Such an accumulation of products in the artificial media of cultivation may perhaps be an important factor in the loss of virulence which seems to result from artificial cultivation, as compared with organisms derived direct from infected soils. From the strictly practical point of view, however, the problem is to determine the conditions under which a further supply of nodule-organisms—such as are now available—would be undoubtedly beneficial to the plants. I am inclined to think that a special study of these conditions is necessary for each type of Leguminous crop; Wollny¹ has recently shown that on chalky soils yellow Lupins and *Serradella* do not react to infection of the soil with nodule-organisms, but that a considerable increase in their development is seen if they receive a supply of easily assimilable nitrogen compounds; and as another example, I may repeat my former statement that for peas grown upon

¹ Wollny, 'Versuche über die Wirkung des Nitragins.' Centralbl. für Bakt. u. Parasitenkunde, 1899.

ordinary garden soil, peat, clay or loam inoculation with 'Nitragin' is useless and superfluous, whilst upon gravelly soils a small increase in crop results from its use.

These unfavourable results with 'Nitragin' lead to the conclusion that the explanation of the problem of the nutrition of Leguminous plants does not depend on the mere presence or absence of the nodule-organisms, but that their effect on the host is directly controlled by the conditions biological, physical and chemical, existing in the soil at any given time, so that it is from bacteriology and chemistry that we may expect to receive great help in the solution of this difficult problem of modern agriculture.

THE BOTANICAL LABORATORY, CAMBRIDGE.

May, 1901.

Cordyceps ophioglossoides (Ehrh.).

BY

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With Plate XXVIII.



MOST of the species of *Cordyceps* are well known to be parasitic upon insects, particularly upon caterpillars. *C. ophioglossoides*, however, together with *C. capitata*, is parasitic upon various Tuberaceae. *C. ophioglossoides* occurs upon *Elaphomyces granulatus*, *muricatus*, and *variegatus*. Masee, in his 'Revision of the Genus *Cordyceps*¹', separates these fungus-inhabiting species from *Cordyceps*, and places them in a distinct genus, *Cordylia*, restricting *Cordyceps* to the insect-inhabiting species. The character, however, seems hardly to be of generic importance. An account of the relationships and distinctive characters of the genus is given in Masee's paper.

The stroma is much like that of the other species of *Cordyceps* (Pl. XXVIII, Fig. 1). It is upright and club-shaped. It is composed of a stalk about 3 inches long and $\frac{1}{8}$ inch thick, and of a head from 1–1 $\frac{1}{2}$ inches long, thicker in the middle and tapering to a somewhat obtuse end. The stalk has a smooth surface and is firm and fleshy in consistency; the head is covered with numerous small papillae, at the tips of which the perithecia open.

¹ Ann. Bot., 1895.

The material with which this research has been carried on was given me by Prof. H. Marshall Ward, who collected it in the autumn of 1900 in Scotland. The tubers upon which it was parasitic were those of *Elaphomyces variegatus*. The greater part was fixed in ordinary methylated spirit, some part in Kieser's solution. The chief stains used were Delafield's and Heidenhain's haematoxylin. I also tried Flemming's and Hermann's safranin-gentian-violet double stains, but the material was not fixed well enough to permit the use of these with advantage.

STRUCTURE AND DEVELOPMENT OF ASCI AND SPORES.

Unfortunately all of the material at my disposal was mature; none of it showed any young stages in the development of the stroma or perithecium.

The cavity of the mature perithecium is roughly egg-shaped (Fig. 2); it passes above into a very narrow neck, and this opens at the top of small, flattish papilla.

The general mass of tissue of the fertile part of the stroma is very loose: it would appear to have been pulled apart by the growth, during the development of the perithecia. The outermost layers, however, are composed of closely woven hyphae, which run for the most part parallel to the short axis of the stroma and transversely to the long axis.

The wall of the perithecium itself is also composed of closely woven parallel-running hyphae. This gives the perithecium the appearance of being an invagination of the surface layers of the fertile stroma.

The hymenium occupies the basal part of the perithecium-cavity: the hyphae of which it is composed are closely packed and run parallel to the long axis of the perithecium.

In a mature perithecium the cavity is occupied by a large number of very long cylindrical asci in all stages of development. This continuous development of asci alone rendered it possible for me to obtain different stages in the formation of the spores. When mature, the asci extend up to the neck of

the perithecium ; young ones are continuously being pushed up between the bases of the older ones.

The youngest ascus I have observed is shown in Fig. 3, which represents the upper two-thirds of the ascus. It contains already about half a dozen nuclei, irregularly distributed. The protoplasm is finely granular, and stains darkly with haematoxylin ; a cap is present even at this stage. I have obtained all stages intermediate between this and the mature ascus.

The nuclei divide repeatedly, and the resulting nuclei distribute themselves quite irregularly in the protoplasm of the ascus. Successive stages in this process of division are shown in Figs. 5 *a*, *b*, *c*, 6, 7, and 8. Figs. 5 *a*, *b*, and *c* show some of the nuclei in the process of division. I was, of course, unable to observe any karyokinetic figures. The protoplasm throughout these divisions remains finely granular, and continues to stain darkly with haematoxylin. During this period of nuclear division the ascus increases slightly in breadth and considerably in length.

At the end of this period we have then the long, narrow ascus, tapering towards the base, and with a well-marked cap (Fig. 12) stretching the greater part of the length of the perithecium. It is somewhat twisted, so that it is rare to get any considerable length of an ascus in one section, even though the section be median to the perithecium ; moreover, the large number of asci in a perithecium renders it almost impossible to piece together the whole of any single ascus from a series of sections. The protoplasm is dense, and shows no traces of divisions. Scattered irregularly about in the protoplasm are immense numbers of small, roundish nuclei (Figs. 7 and 8).

After divisions have ceased, the nuclei become arranged in rows longitudinal to the long axis of the ascus. Owing to the large numbers of nuclei and the narrowness of the ascus, this does not involve any great change of position for the nuclei, as may be seen from Fig. 8. Eight longitudinal rows are formed, of which three are usually seen in section.

Following this rearrangement, longitudinal lines of division

appear in the protoplasm of the ascus (Fig. 9), dividing this up into eight filiform ascospores; these are thus multinucleate from the beginning. The ascospores soon become rounded off and acquire a wall, not, however, till further divisions have occurred. In sections transverse to the ascus, seven outer ones are seen surrounding a single inner spore (Fig. 14 *a*).

Immediately following, indeed almost contemporaneous with, the longitudinal divisions, transverse lines appear, dividing each ascospore into a large number of cylindrical, uninucleate sporidia (Fig. 10). Stages such as that shown in Fig. 9 are very rare. The divisions seem to occur simultaneously throughout the ascus.

The sporidia, once formed, become separated, and each acquires a wall; they still, however, remain eight-ranked (Figs. 11, 14 *b*). The ascus becomes considerably broader and rather longer during this process (compare Figs. 14 *a* and *c*), and its wall becomes very fine and almost invisible. The sporidia become rounded at the ends; when ripe they are cylindrical-ellipsoidal in shape, about $4\ \mu$ long by about $2\ \mu$ broad. During the ripening the protoplasm becomes clear and free from granules, and stains much less darkly; the nuclei become somewhat larger, the contrast between young and ripe asci is thus very marked. During this time also the walls of the sporidia become changed chemically, and are probably rendered harder. They now resemble the caps of the asci: this was very clearly shown in sections of material fixed in Kieser, which were stained with Hermann's safranin-gentian violet; the walls of the ripe sporidia and the caps of the asci were coloured dark violet, while the walls of the younger asci and spores were pink (the nuclei and protoplasm were not stained). Fig. 13 shows part of an ascus with ripe sporidia: these are probably contorted in fixing.

ANATOMY OF STERILE PART OF STROMA.

The sterile part of the stroma shows nothing of special interest. The central mass consists of closely interwoven, septate, narrow hyphae, with thin walls, running quite irregu-

larly. The outer layers are composed of larger hyphae, with thicker walls and less cell-contents than the central ones: these outer hyphae are interwoven as are the central ones, but they are more regular; their general course is parallel to the long axis of the stroma (Figs. 15 and 16 *a*). There appear to be no special conducting or 'laticiferous' tubes present.

CONNEXION BETWEEN CORDYCEPS AND ELAPHOMYCES.

When sections were made of the lower part of the *Cordyceps* stroma, they were found sheathed with a layer of large, rather thick-walled hyphae belonging to *Elaphomyces*. Lower still, masses of these hyphae appeared as islands in the central mass of the *Cordyceps* stroma. These hyphae were considerably larger even than those of the outer layers of the *Cordyceps* (compare Figs. 16 *a* and *b*): they were much more loosely arranged, and their walls always stained more readily than those of the *Cordyceps* hyphae, so that there was little difficulty in distinguishing between the two sets.

The relation that the *Cordyceps* thus bears to the *Elaphomyces* tuber has a broad resemblance to that borne by the young sporophyte of an Archegoniate plant to the gametophyte, which is essentially a parasitic one. The basal part, then, of the *Cordyceps* stroma may be compared to the foot of the *Anthoceros* or Fern embryo, or to the absorbent cotyledon of a grass or palm in the endosperm. In all my material, which only showed mature *Cordyceps* stromata, very little of the original *Elaphomyces* tuber remained: it has been exhausted, and the tissue replaced by the advancing 'foot' of the *Cordyceps*.

If the *Elaphomyces* hyphae be more closely examined, they are found to be interwoven with smaller, thin-walled hyphae of *Cordyceps*. It seemed possible that the parasitic hyphae developed haustoria, by means of which the nutriment was absorbed from the host. I spent accordingly a considerable time in examining a large number of sections, taken in all directions and from several specimens, in order to determine, if possible, the presence or absence of haustoria.

I was, however, unable to distinguish anything of the nature of a definite haustorium. Though, of course, in such a case negative evidence is of little value, still it is hardly probable that if haustoria were present in any number, I should never have observed them.

Unions, however, do exist between the two sets of hyphae, and these are fairly frequent. Where they occur, the two adjoining walls fuse and become considerably thinner; the thickness of the partition is certainly not more than that of the walls of the *Cordyceps* hyphae themselves (Figs. 17, 18, 19, 20). In some cases, moreover, the *Cordyceps* hypha projects into the cavity of the other (Figs. 21, 22, 23), in which case the union has the appearance of a rudimentary haustorium.

Whether or not these hyphal fusions are sufficient for the nutrition of the *Cordyceps* I cannot say: they suggest again the comparison with a Phanerogamic embryo parasitic upon the endosperm, or the sporophyte of a fern upon the gametophyte. Possibly an examination of the *Elaphomyces* tuber in the earlier stages of the attack, and while the *Cordyceps* stroma is still actively growing, would give more definite results.

THE MYCORHIZA OF ELAPHOMYCES VARIEGATUS.

Elaphomyces has long been known as one of those Fungi which form a mycorrhiza with the roots of Conifers. Reess¹ has described the mycorrhiza on the Pine roots.

Among my material was a rootlet of *Pinus*, bearing a coral-like mass of secondary and tertiary rootlets forming the mycorrhiza; this was still in connexion with a part of the rind of an *Elaphomyces* tuber, so that no doubt could be entertained as to the identity of the mycorrhiza Fungus. One specimen which is now in the Botanical Museum at Cambridge shows the whole connexion between the *Cordyceps ophioglossoides*, *Elaphomyces variegatus*, and the mycorrhiza on the Pine root; it is probably almost unique.

As my observations differ somewhat from the account given

¹ Bibl. Bot., 1887.

by Reess, I thought it advisable to give a short account of the mycorrhiza-structure, so far as could be made out from the material at my disposal.

Reess describes the mycorrhiza as being ectotrophic and says, at all events for the young stages, that the Fungus extends into the root only a distance equal to the thickness of the fungus-sheath outside. He describes these internal hyphae as being intercellular, and as giving rise to peculiar mulberry-like ingrowths into the cell-cavity of the cortical cells. He is in doubt as to whether these structures are haustoria, or merely hypertrophies of the cell-wall caused by the action of the intercellular hyphae; he seems on the whole to favour the former view, at any rate in some cases, while in others the growths appear to show no lumen and give the reactions of ordinary parenchyma-membranes, while their connexion with the intercellular hyphae is doubtful. These structures are described as being conspicuous and of frequent occurrence.

My observations show that *Elaphomyces*, as a mycorrhiza Fungus, is rather variable in its structure.

The structure most frequently found shows in the young stage the ordinary fungus-sheath on the outside of the root, and this is continuous with an intercellular network (Fig. 25) between the cells of the root-cortex. The network in surface view has the appearance of a reticulate thickening of the cell-walls. This structure is that described by Frank¹, for the mycorrhiza on the roots of Cupuliferae, but in his case only the outermost layer of cells of the root was concerned. In my sections the whole of the root-cortex was seen to be penetrated in this way by the Fungus (Fig. 24).

One very characteristic feature of this mycorrhiza is the action of the Fungus on the cortical cell-contents. These are transformed into (apparently) slimy, deeply staining masses, which contain fat (Figs. 25, 27, 28, 29). This change takes place right through the cortex of the root. Sometimes a large, more or less disorganized nucleus is present in the mass.

¹ Bot. Zeit., 1885.

* In none of my preparations showing this intercellular network was there any sign of intracellular hyphae or of haustoria. I may say now that in no case have I ever seen anything of the peculiar mulberry-like ingrowths, figured and described by Reess.

The next older stage of this form of the mycorrhiza is shown in Fig. 26. On the outside is the pseudoparenchymatous fungus-sheath, which has invaded in parts the root-tissue—the outer layer of cells of the root is quite broken up, leaving only bits of cell-wall surrounded by the fungal network. Following this fungus-sheath we get the root-cortex: this no longer shows the intercellular network of internal hyphae; the cells are separated by large empty spaces; they are filled with the slimy, dark-staining masses mentioned above. In nearly every case the walls of these cortical cells are beginning to break down. In the centre of the section we get the feebly developed stele, which appears quite healthy and unaffected by the Fungus.

The oldest stages have a very peculiar appearance (Figs. 27, 28). As before on the outside we get the fungus-sheath quite healthy and unchanged. In the centre of the section, also healthy and unchanged, is the stele. But between the fungus-sheath and the stele we have simply an almost homogeneous mass of slimy, disorganized cell-contents, in which we are still able sometimes to trace the outlines of the original cortical cells. Scattered in this we get broken bits of cell-walls and some few nuclei.

Stahl¹ has recently treated afresh the whole question of mycorrhiza; he reviews all previous work and himself supports Frank's view that in all cases the mycorrhiza is a symbiotic union between the Fungus and the host root. He regards the Fungus as the dominant partner, which has forced itself on the host by cutting off the food-supply, and so compelling the higher plant either to compete with it by means of an extensive root and leaf-system, or to submit to an alliance.

Certainly in the union between *Elaphomyces* and the Pine

¹ Prings. Jahrb., 1900.

root, the Fungus would seem to be dominant. My observations do not show whether or not the Pine gains any compensating advantage for the loss which it undoubtedly sustains through the presence of the *Elaphomyces* hyphae. Certainly the roots have the appearance of suffering from the attack of a true parasitic Fungus, and it is difficult to imagine such a cortex as shown by them being of any service in the transport of nutrient material to the stele.

I must now briefly describe other appearances presented in the Pine roots, by apparently the same Fungus.

When sections were taken of the main rootlet they showed that no external fungus-sheath was present. The root, however, was not free from the Fungus; in many of the cortical cells the cell-cavity was filled with a pseudoparenchymatous network of hyphae (Figs. 29, 30). The outer cortical cells appeared to be first attacked, but the Fungus afterwards extends throughout the cortex, and in many cases the network was to be seen even in the cells of the endodermis. The walls of the cortical cells are fairly thick, some of these cells, as also some of the parenchymatous cells of the stele are filled with dark-staining substance (Fig. 29), similar to that shown by the cells of the cortex in the other roots as mentioned above. There is certainly a considerable difference between these hyphae and the intercellular network of the smaller rootlets, but there seemed no trace of the presence of another Fungus, and I myself am convinced that both sets of hyphae belong to *Elaphomyces variegatus*.

Another appearance presented by this mycorrhiza was only seen by me in one series of sections. It here showed one of the main rootlets of the mycorrhiza, and arising from this a smaller one. Both were covered with a fungus-sheath which was continuous over the point of origin of the secondary rootlet. The smaller rootlet showed the younger stage of the intercellular network. The larger rootlet on the contrary showed nothing of this, the walls of the cortical parenchyma were thickened and quite normal (Fig. 31). But in many of these cortical cells were seen bundles of fungal hyphae

or single hyphae (Fig. 31). These do not agree in any way with the description and figures given by Reess of his haustoria.

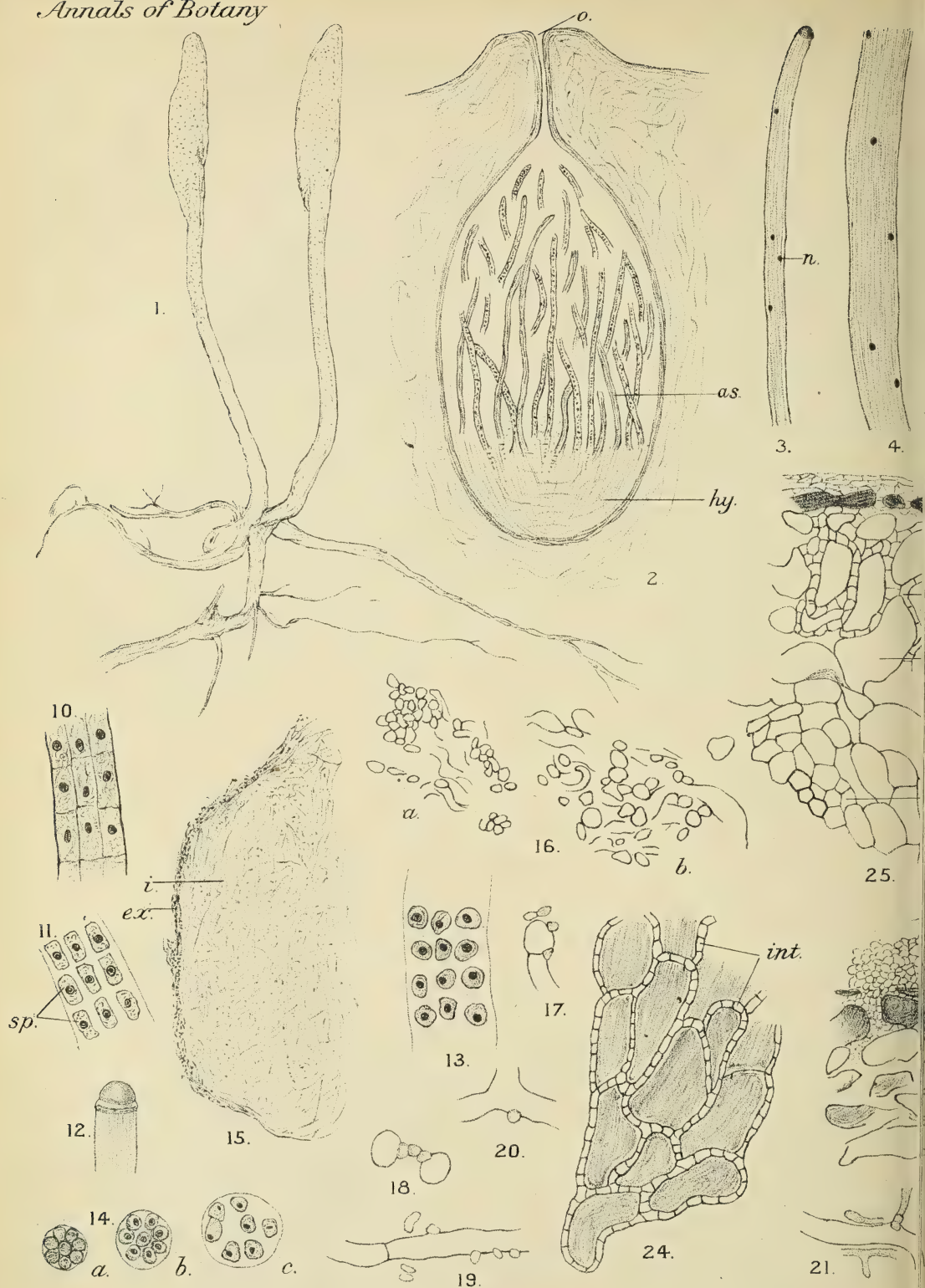
If these three forms of mycorrhiza are really due to the action of one Fungus, and this certainly appears most probable, it would seem to show that *Elaphomyces variegatus* is capable of varying its method of attack, according to the strength of the root attacked; when this is small, with thin-walled cortical parenchyma, we may suppose the intercellular network is sufficient for the destruction of the cortex. On the other hand we must presume that the older roots had not been attacked until the cortical cells had thickened their walls, in which case the formation of the intercellular network was not possible or not sufficient, and so the Fungus resorted to the intracellular method of attack. I offer this merely as a suggestion; it may be, of course, that three distinct Fungi were present.

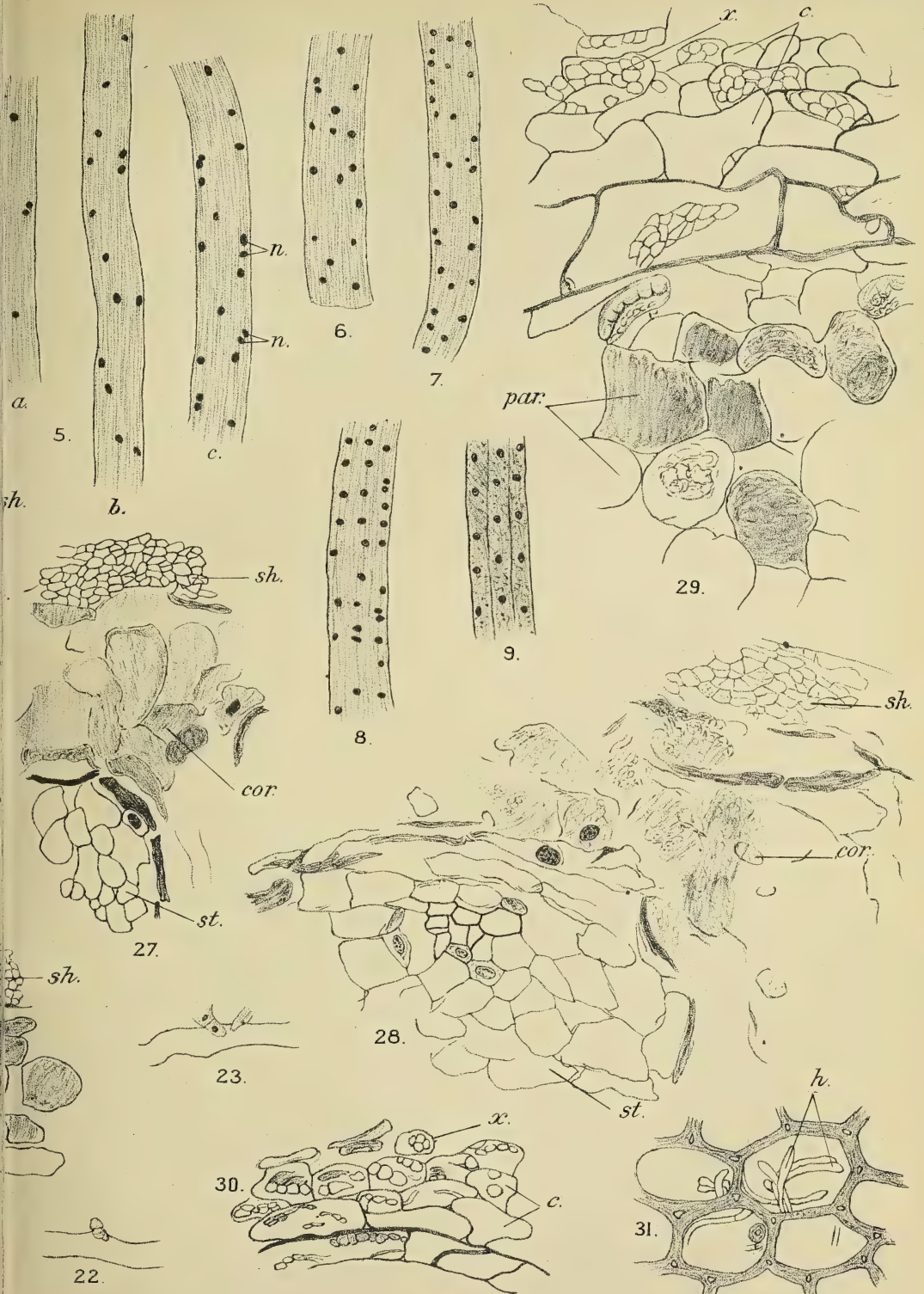
In conclusion I have to thank Prof. Marshall Ward for his kindness, not only in giving me the material with which to work, but also for his assistance while the work was in progress. I have also to thank Mr. R. H. Biffen for much advice and assistance.

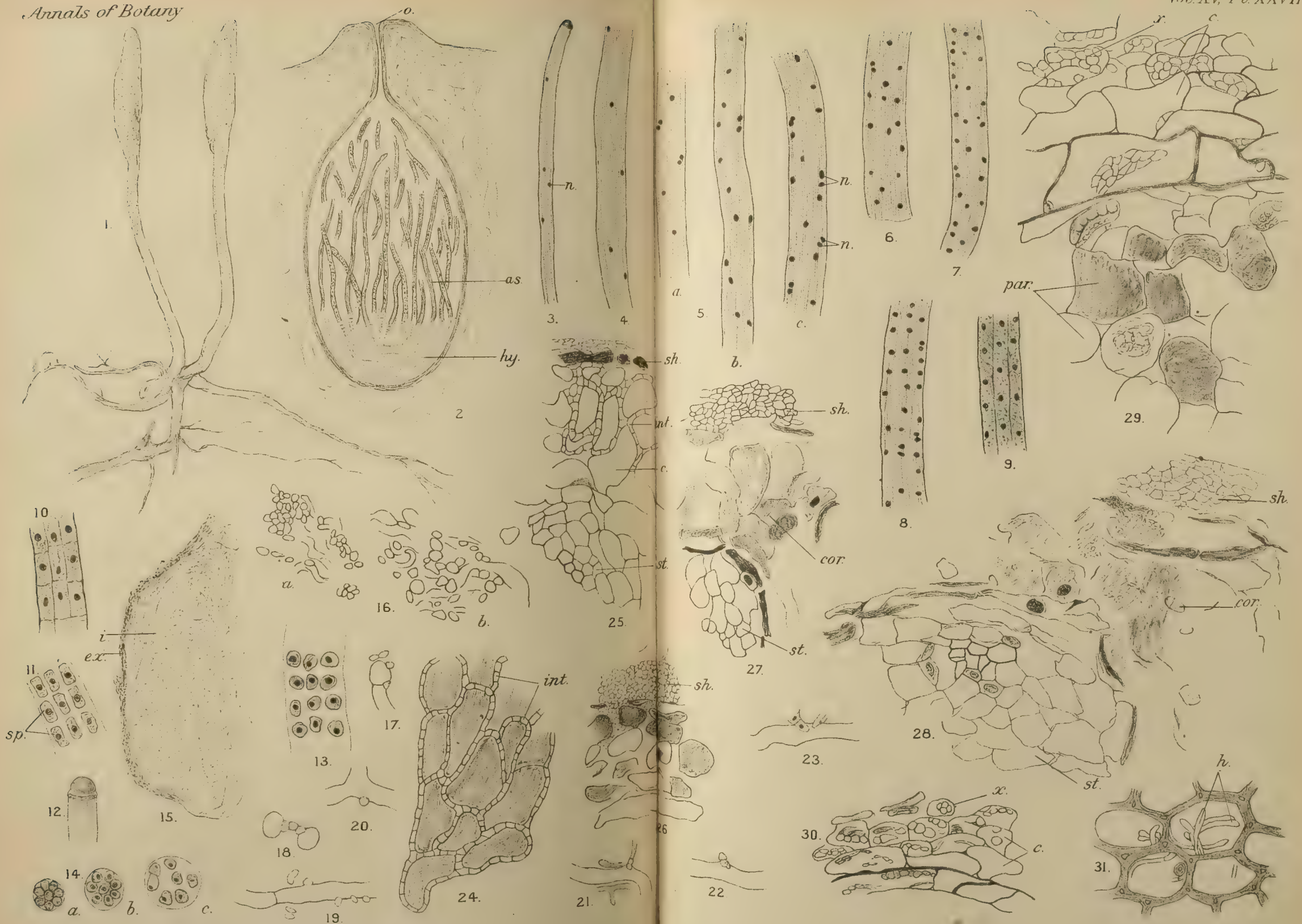
EXPLANATION OF FIGURES IN PLATE XXVIII.

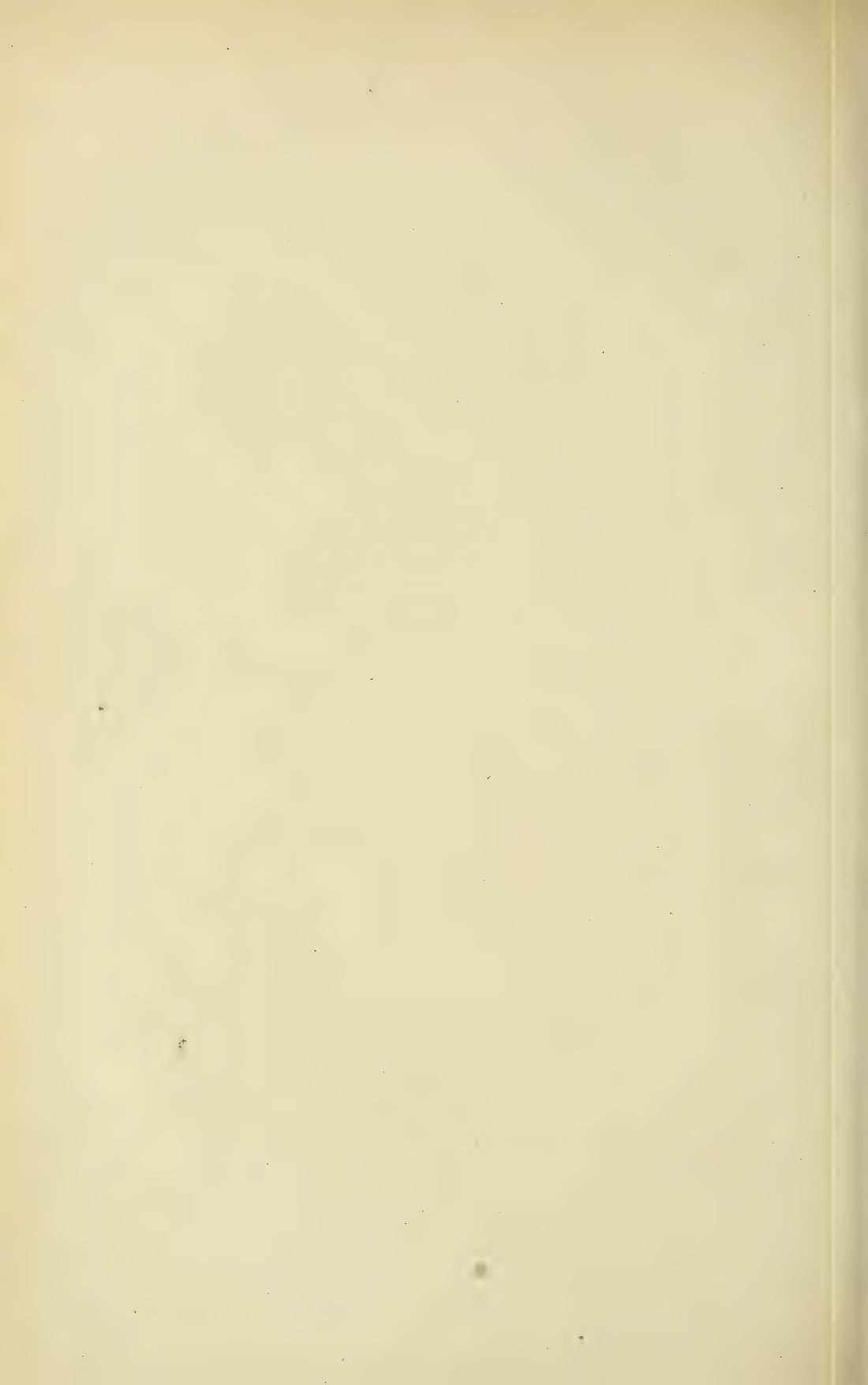
Illustrating Mr. Lewton-Brain's paper on *Cordyceps ophioglossoides*.

- Fig. 1. General drawing of *Cordyceps ophioglossoides*, with two mature stromata.
 Fig. 2. Mature perithecium in median section; *hy.*, hymenium; *as.*, asci in different stages of development; *o.*, opening of neck of perithecium.
 Fig. 3. Part of young ascus in longitudinal section, with very few nuclei (*n.*).
 Figs. 4, 5 *a*, *b*, *c*, 6, 7, 8. As Fig. 3 showing progressive stages in division of nuclei, before divisions occur in the protoplasm.
 Fig. 9. As before, showing longitudinal line of division in the protoplasm.
 Fig. 10. As Fig. 9, showing both longitudinal and transverse divisions.
 Fig. 11. Part of ascus with nearly mature sporidia.
 Fig. 12. Cap of ascus.
 Fig. 13. Part of ascus showing three rows of ripe spores.
 Fig. 14. Transverse sections of asci of various ages.
 Fig. 15. Transverse section of sterile portion of stroma; *ex.*, outer hyphal layer; *in.*, central hyphae.
 Fig. 16 *a* and *b*. Camera lucida drawings with same magnification (about 1000), (*a*) of external hyphae of *Cordyceps*, (*b*) of *Elaphomyces variegatus*.
 Figs. 17–23. Various unions between large hyphae of *Elaphomyces* and the smaller parasitic hyphae of *Cordyceps ophioglossoides*.
 Fig. 24. Part of tangential section of cortex of Pine root, showing intercellular network of hyphae (*int.*).
 Fig. 25. Transverse section of root in same condition as in Fig. 24; *sh.*, fungus-sheath; *int.*, intercellular network; *c.*, cortical cells; *st.*, stele. × 225.
 Fig. 26. Part of cortex of Pine root, showing more advanced stage than Fig. 25. × 225.
 Figs. 27, 28. Sections showing still more advanced stage; *cor.*, remains of cortex. Fig. 27 × 225.
 Fig. 29. Part of rootlet in transverse section, without external fungus-sheath; *x.*, intracellular network of hyphae; *c.*, cortical cells; *par.*, parenchyma of stele.
 Fig. 30. Part of cortex of similar root.
 Fig. 31. Part of cortex of another rootlet, showing bunches of intracellular hyphae (*h.*).









On the Reaction of Leaves to Traumatic Stimulation.

BY

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AND

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With Plate XXIX, and five Figures in the Text.



IN the course of the experiments on respiration which we have been carrying on for some time, we have come across some interesting phenomena connected with the reaction of leaves to experimentally produced injuries. We have followed these out to a certain extent and propose to give now an account of the results.

The vitality of cut-off leaves, kept in the dark but supplied with water, is considerable and much beyond general expectation. Leaves of Cherry-Laurel remain healthy for perhaps fifty days in such conditions, and leaves of Oleander have been kept thus for several months without ever having a chance of obtaining any fresh carbon-nutrient. The behaviour of the respiration under these conditions we shall treat of elsewhere: here it may be noted that Oleander leaves invariably put out vigorous crops of adventitious roots from the stump of the leaf-stalk under these conditions, and that

removal of the roots may be followed by a second crop and even a third. Cherry-Laurel leaves, however, never produce roots, but they develop a callus from the cut surface of the leaf-stalk which forms a well-marked pad over it.

Such recuperative processes indicate considerable vitality, but the remarkable activity of the leaf that can be induced by injury even after some weeks of starvation shows that the leaf is really in full vigour.

We will confine ourselves in this paper to the case of the Cherry-Laurel (*Prunus Laurocerasus*, garden var. *rotundifolia*), and at first to its behaviour when the leaves used are such as have been cut off and are being kept in the laboratory in a beaker, with the cut stalks in water. A very loose lid is placed on the vessel so that the air surrounding the leaves is kept moist. If patches of cells of these leaves be killed—and this conveniently becomes evident by their turning brown very quickly—the surrounding sound tissues always react by cutting off and exfoliating the injured patch, so that it drops, without any external assistance right out of the leaf, and a hole results. The line of exfoliation follows the outline of the injury with some precision but at a certain distance from it, and so any number of holes of any desired outline can be brought about; the leaves 2, 3, 5, 6, in Plate XXIX, are bizarre examples of the effect.

The healing of wounded leaves has been previously described for several cases, but we can find no description of the process of exfoliation which we have observed, and of which we may now give a detailed account.

I.

If a clean cut be made through the substance of a Cherry-Laurel leaf with a sharp knife no healing reaction follows. In ever so many cases ten or more incisions have been made in each half of the lamina, at right angles to the midrib and extending from the midrib right to the edge of the leaf. A leaf thus cut into some twenty segments, united only by means of the midrib, will if protected from too great dryness

in the way that we have indicated remain alive, healthy, and turgid for a month or more, although all the intercellular spaces are thus thrown into uncontrolled communication with the external air. The cells that are actually cut through of course die and some three layers of adjacent cells may perish but never a sufficient number to produce a brown edge visible to the naked eye.

If, on the contrary, a sufficient number of cells be killed to produce a visible brown margin to the cut, then the cutting-out reaction will follow. All the methods of killing tried, including rupturing the cells by a heavy blow, and contact with a hot iron, provoke the same reaction. The neatest method that we have employed, now to be described, may seem a rather indirect one, but we were led to it in the course of our respiration experiments. If a leaf, cut in any way, be injected with water under the air-pump, and if afterwards for a few minutes the water be rapidly dried out of it—as in vacuum—then the cells all round the edges of the cuts are disorganized and killed and quickly turn brown for a distance of one to three millimetres according to the duration of the treatment. The brown margin that results is fairly uniform all over any given leaf and the cutting-out reaction follows with certainty.

The first sign of the reaction is visible five or six days after the leaves have been put back in a vessel with their stalks in water; at least this holds good for experiments made in April, May, and June at laboratory temperatures. This indication consists of a fine sharp line running all round the dead region at a distance of one to three millimetres. When the leaf is viewed against the light, this line is seen to be quite translucent and strongly contrasted against the rest of the opaque lamina.

On cutting sections, at the first glance there appeared to be no internal corresponding change, but it was soon seen that the spongy parenchyma in the track of the line had grown and divided so as to entirely block the intercellular spaces and so had produced a local translucency, just like that produced by filling up the intercellular spaces of a leaf with water.

The fourth of the photographs in Plate XXIX shows this stage, but only as seen by reflected light, when the line is not nearly so obvious. In the photograph there can be distinguished (1) the original cuts between the leaf-veins made with a sharp knife, (2) the brown fringes round the edges of the cuts produced by cells that have been killed by rapid drying, and (3) the fine sharp occlusion-line running round each killed area.

After a few more days the epidermis on both surfaces of the leaf will be found to be cleanly split through, all along the track of the line. The splitting extends steadily through the substance of the mesophyll and in another week or two the separation will be complete, the circumscribed areas will drop out, and leave such a leaf as is shown in the fifth photograph—a perfectly healthy leaf which has thrown off all the dead portions and has its edges closed in again all round.

We have worked out roughly the histology of the separation-process by cutting sections through the lamina at right angles to the track of the translucent line. An absciss-layer arises

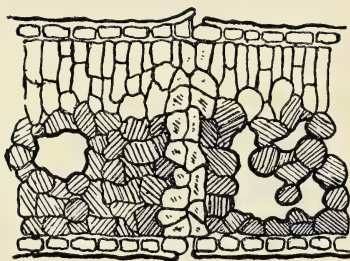


Fig. 4.

by the division of a single row of cells running from one epidermis to the other through the occluded spongy parenchyma and the palisade-tissue. As soon as the cells along this row have divided into two the cuticle seems to crack sharply across and the contiguous epidermis-cells in the region of the line become separated from

one another without themselves taking any part in the division. Fig. 4, which is rather schematic, shows this stage, and it is to be noticed as a constant character that the absciss-layer does not form in the middle of the solid occluded portion of mesophyll, but always on the side towards the dead cells.

The new cells of the absciss-layer are poor in cell-contents, and the two layers of cells soon round off and so separate

from one another (Fig. 5), till the two portions of leaf are quite free. No further histological change takes place in the piece of leaf that falls off, but on the edge belonging to the main leaf these new cells go on multiplying parallel to the new edge, till we get the appearance of a meristem with a number of tiers of swollen cells beyond, generated by its activity and giving the edge a velvety look. These

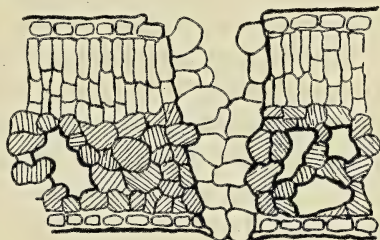


Fig. 5.

cells have very thin walls but are cuticularized. Fig. 6 is made from a preparation in strong sulphuric acid; the mesophyll has all been dissolved and the cuticularized pad of callus-like cells is seen to be attached to the cuticle at both surfaces of the leaf, so as to close in and protect the mesophyll as effectually as before.

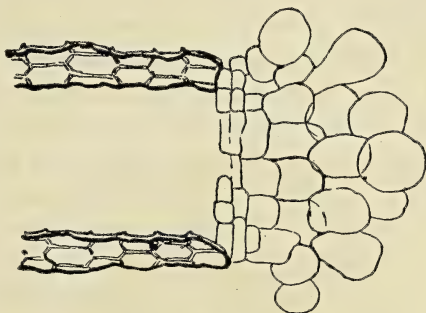


Fig. 6.

Some instructive variations of the cutting-out process must now be considered. If a number of dead areas be produced close together there may not be sufficient sound tissue between them for the line of occlusion and the absciss-layer to be formed at the proper distance from the dead cells. The line of demarcation then sweeps round and includes several contiguous dead areas in one contour.

The first photograph (Plate XXIX) shows a good case of this, as a line of occlusion envelops seven parallel strips of dead tissue and only attempts to pass between the upper two which are rather further apart. The second photograph shows the same leaf at a later stage with the whole patch of living

and dead tissue nearly exfoliated in one large piece. In the third photograph those circular dead patches which are well separated are each surrounded with an effective absciss-layer, but in the upper part of the leaf four such dead spots are close together and they are being cut out by one common absciss-layer. The appearance of this leaf is rather complicated by the large round hole in the middle of each dead area. In this case, circular areas of cells were killed by

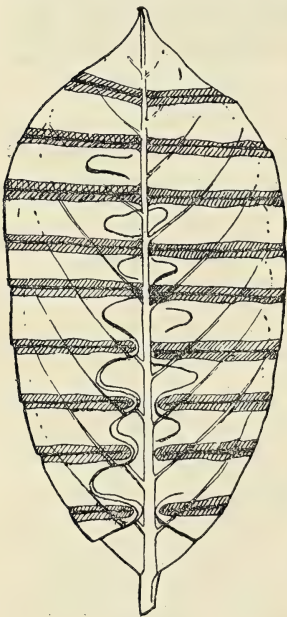


Fig. 7.

a round piece of hot iron, and subsequently, but this is of no present significance, the centres of these were removed by a cork-borer of smaller diameter than the piece of iron. The circular absciss-layer is formed in the sound tissue round the patch and so ring-shaped stretches of tissue are being exfoliated, and are at present attached by one or two points only. Where the absciss-layer has to pass through a vein the processes take place more slowly, but the largest of the secondary veins may be effectively dealt with, as the sixth photograph shows, and in this also the pieces are dropping out. We have not observed any case of the absciss-layer succeeding in passing through the midrib. It follows then that if a

strip of dead cells extends across the leaf from the edge right up to the midrib it cannot be completely surrounded with an absciss-layer and thrown off. It becomes interesting to see what reaction will then take place.

The leaf shown diagrammatically in Fig. 7 furnishes a case in point, and it merits detailed consideration. The lamina was cut and the marginal cells killed by rapid drying as in the other cases, but the treatment was more prolonged, and very broad dead brown margins border all the cuts. In the

upper part of the leaf these dead strips extend right to the midrib, but in the lower part they just fall short of it. The position of the resulting absciss-layers is shown by the curved black lines, and everywhere, even in the upper part where they outline isolated tongues, one in each sound compartment of the leaf, the actual separation of tissues has taken place as definitely as if produced by a sharp scalpel.

Here the absciss-line no longer runs a parallel course with the edges of the dead area. In the few places where it is approximately parallel it stands much further off than in the previous cases, which we think is related to the more extensive injury. Except at one point the absciss-lines do not run out to the edge of the leaf, but they curve round in the middle of the sound strips of tissue and join one another to form larger or smaller tongues, retaining a small amount of tissue inside their curves and leaving large masses of green healthy tissue outside to be exfoliated with the dead. This certainly has the appearance of being a radical surgical treatment of injuries which are too extensive to be dealt with in detail, and we believe it really has this sort of significance.

In the upper part of the leaf even this attempted radical cure fails, because the dead strips extend right to the midrib and so in no way can they be thrown off. In the lower part of the leaf there are very narrow tracts of sound tissue left round the central ends of the dead strips, and in all these cases the absciss-line extends along them from one tongue to another and so the dead tissue is all cut off, as on the left half, below, though with great sacrifice of sound tissue as well. The leaf, by a continuation of this treatment, would be reduced to a midrib bearing a few almost isolated tongues of sound tissue.

Below the lowest injury the absciss-line makes its way to the edge and a quarter of the leaf is hanging, attached only above.

It is further very interesting to note that where the absciss-line passes along the narrow bridges of sound tissue between the successive dead strips and the midrib it has to be formed extremely close to the dead cells, and not at the usual con-

siderable distance. This indicates again a departure from the usual type of reaction, a special effort—so to speak—made to secure the exfoliation of all these extensive dead masses.

It is difficult to resist the conclusion that the leaf is reacting as an autonomous whole and modifying the usual parallel absciss-line, to suit the special difficulties of its condition. The behaviour of this leaf seems to us to strongly support the view, suggested by the great vitality of these isolated leaves, that the solidarity and autonomy of a single leaf may be considerable.

II.

All the leaves described above were kept moist in beakers, as has been stated. When leaves attached to the shrub growing in the open were experimented upon, the reaction

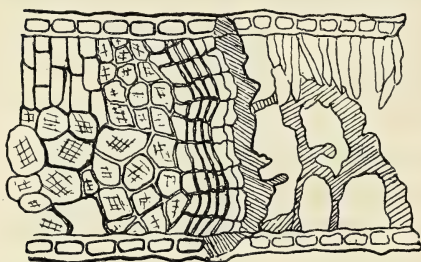


Fig. 8.

to incisions was found to be quite different, and it never took the form of the exfoliation which we have just described.

If cuts be made with a scalpel through the leaves of Cherry-Laurel, while still on the plant, they quickly become bordered with a very narrow edge

of dry brown cells, and in a week or two a translucent line may form parallel and very close to the brown edge of the cut. This is an occlusion-line of normal structure, and a line of active cell-division forms in it on the side towards the cut edge. *No trace of separation is found*, however, between the daughter-cells that arise from it, but they remain compactly adherent and division is continued, so that a periderm of several layers of cork results, as is shown in Fig. 8.

Let us now turn to the records of previous workers, before we consider the problems that these reactions suggest. There

is no occasion for us to consider the literature of wound-healing in general, as a good account was given of it by Massart ¹ a few years ago. Confining ourselves to leaves, we find that many different reactions have been observed on leaves *in situ* as a result of spontaneous, accidental or experimental injuries. Collating the chief papers, those by Bretfeld ², Frank ³, and Massart ¹, we may distinguish at least five different cases, none of them involving formation of an absciss-layer.

(1) In *Camellia* and in many marsh plants it is said that there is no reaction, and that the killed tissues dry up to form a scar over an injured surface ⁴.

(2) In *Nuphar* and other aquatic plants when the large internal spaces are laid open by injury there takes place a filling up of the lacunae by growth of the cells which border them ⁵.

(3) In *Leucojum* something similar takes place, but the outgrowing cells form long almost unseptate tubes, tightly packed and capable of uniting with the cells growing out from the opposite side of a cut, to make a union of the fracture. Frank considers this to be a primitive form of callus, and the cells, though thin-walled and of sparse contents, are cuticularized and resist sulphuric acid ⁶.

(4) In *Cornus* ⁷ and *Hoya* ⁸ we get septate outgrowths of the mesophyll-cells to form a compact mass of isodiametric cells without intercellular spaces, which Frank regards as a typical callus.

(5) In succulent and in some other leaves several layers of corky cells may arise by division in the mesophyll-cells parallel to the surface of the wound. This takes place in quite the same way as the well-known new formation of cork below the wounded surface of a potato ⁹. No increase in bulk of

¹ Massart, La cicatrisation chez les végétaux. Méms. couronnés de l'Acad. roy. de Belgique, T. 57, 1898.

² Bretfeld, Vernarbung u. Blattfall, Pringsheim's Jahrb., vol. xii, 1879.

³ Frank, Die Krankheiten der Pflanzen, Breslau, 1895, Bd. i; cf. also the section on the same subject in Schenk, Handbuch der Botanik, Bd. i.

⁴ Bretfeld, loc. cit., p. 139.

⁵ Massart, loc. cit., p. 41, Fig. 43.

⁶ Frank, loc. cit., p. 65, Fig. 12.

⁷ Frank, loc. cit., p. 67, Fig. 13.

⁸ Massart, loc. cit., p. 57, Fig. 54.

⁹ Bretfeld, loc. cit.

the original cells takes place, only septation, and this is the typical 'wound-cork' of Frank ¹.

(6) The exfoliation by an absciss-layer described by us in *Prunus Laurocerasus* forms a sixth type of reaction.

Here then we have six modes of reaction, apparently very distinct from one another, yet on reflection there is no doubt that the leaves of the Cherry-Laurel show processes that suggest comparison with each one of them. The absence of structural reaction; the occlusion of spongy parenchyma; the outgrowth of bulging cells at the edge after exfoliation; the development of the solid pad of tissue in which the meristem forms; and the periderm-formation in attached leaves, are five processes, having many points in common with the first five types of reaction respectively. In addition there is also the exfoliation-process.

The variations in the reaction seem to be correlated with the nature of the surrounding conditions, and it is clear that no further progress can be made in sorting-out these different processes without bringing the problems from the field into the laboratory where the conditions can be carefully controlled. For this to be successful, leaves of marked vigour and vitality are essential, and those of the Cherry-Laurel promise well in this respect. We hope to carry further the investigation of the controlling effects of external conditions.

It may be mentioned here that only fully grown leaves have been used in all cases, and as most of the work was done in the early summer, the leaves used were necessarily those of the previous year: in July, however, the newly matured leaves give similar results. Very young leaves possess a considerable power of regenerating lost tissues, as distinguished from the carrying out of mere healing processes, and in them the epidermis may divide as well as the mesophyll and new typical epidermis may be generated. This normally happens with these fenestrated leaves which develop large perforations as they unfold ².

¹ Frank, loc. cit., p. 60.

² Massart, loc. cit., p. 55, Fig. 47, also p. 30.

Being able to obtain the exfoliation-effect with certainty in the laboratory we have looked out for signs of it in the field, and have seen a number of specimens illustrating it, although it has never occurred in response to our experimental lesions. In natural specimens the green sound tissue on the distal side of the absciss-layer dries up quickly and turns brown before the piece drops out, so that the separation appears to be taking place at the exact edge of a dead patch as if it were only a mechanical rupture between dry and living tissue.

In the laboratory, further, we have occasionally found that leaves which have remained for a long time with their stalks in water may proceed to exfoliate small areas which happen to have become injected with water; and rarely, small exfoliations take place for which there is no obvious cause. We hope that further experiments will throw light on these cases.

III.

Finally, we may just touch on two or three problems and give a few tentative reflections that suggest themselves, in relation to the contrasted reactions that have been noted, and to the biological significance of the various processes.

(1) Occlusion of the spaces in a spongy parenchyma would seem to be a necessary preliminary to the formation of a definite line of meristem in that tissue. Is this the whole significance of the occlusion-line, or is the process of direct value as a reparative one, closing in again the internal spaces of the leaf?

Though occlusion has been described as one of the types of wound-healing, yet we incline to the other view here, as it takes place very locally and is closely linked to the subsequent activity, and occurs so well in the leaves kept in damp air, which do not require for their continued sound health to have their spaces occluded at all.

(2) The fact that leaves do not react to a clean incision, which kills only a minimal number of cells, seems to us to be of the first importance. We have several times found that the disorganization of rapid drying may fail to kill an appreciable

number of cells at just a few places along the edges of the cuts in a leaf, as shown by the absence of brown colour: just at these places and at these only, as we have photographs to prove, the occlusion and abscission is very long delayed or may not take place at all.

We conclude therefore that where the factor of excessive dryness does not come in, the reaction of the leaf is directed against its connexion with the dead cells, and not against the free communication of its interior with the open air. Speaking teleologically, the leaf is presumably protecting itself against the risk of infection which might get a start in dead cells which are kept moist by diffusion from living ones. The cases in which abscission follows fatal local heating without destruction of tissue also point to the same explanation.

When leaves freely cut open are left exposed to the dryness of the summer atmosphere, cells at the edge are soon killed off by natural drying, and we afterwards become unable to say which factor it is that provokes the subsequent reaction.

(3) Another problem lies in the causational difference between periderm-formation and abscission. Both processes seem to arise at corresponding spots in the line of occlusion, and both start by division of a more or less definite line of cells into daughter-cells. What are the factors that decide whether these cells shall dissolve the middle lamella between them, round off and separate, or whether they shall remain united as a phellogen and a first layer of cork?

The relative dryness of the surrounding air appears to be the essential cause, but we do not know whether isolation of the leaves from the parent plant has any effect. Many observers have noted that dryness promotes cork-formation in various cases, and Massart¹ showed ingeniously that wounds opening to the atmosphere provoke more reaction than similar ones opening only internally into the natural hollow of a herbaceous stem, and also that only the layers exposed to external air became suberized. Massart² also quotes some very suggestive but inverse observations as to the effect of

¹ Massart, loc. cit., p. 46, Figs. 48-50.

² loc. cit., p. 58.

prolonged exposure to damp air on parts that normally form only cork. Thus in damp air the lenticels of *Sambucus* will grow out into tufts which consist of long multicellular filaments produced by the underlying phellogen ; and even the phellogen under the leaf-scars on branches of *Populus* may react in the same way, although many layers of cork have been previously produced by it. The intumescences on *Hibiscus*, worked out by Miss Dale¹, seem to belong to the same category in their causation.

This stimulation of a phellogen to grow out into filaments seems somewhat analogous with the stimulation of a meristem, so that the sister-cells become turgid and tend to round off from one another as in abscission. As soon as an abscission-line is formed in a dry atmosphere the parts of the leaf cut off outside it dry up, and so the line appears to have arisen close to the dead patch and not back in the sound tissue as it has done in reality.

(4) The distance from the lesion at which the reaction takes place seems to be constant for a given amount of injury, as the photographs all show, and to be further back the greater the killed area, but other factors also come in, as with the leaf in Text-Fig. 7. A curious case is given by Massart² in which a reaction takes place at several centimetres from the seat of the injury. If an internode of *Impatiens Sultani* be cut through at the top no change takes place there, but a reaction takes place at the very bottom of the internode, where abscission takes place—as in leaf-fall—and the whole internode is thrown off. Also with many leaves, if the midrib be destroyed, the leaf becomes cut off at the normal position at the base of the petiole and a leaf-scar is formed there.

(5) Finally, the addition of the new phenomena which we have described to the previous stock of known reactions to traumatic lesions complicates very much the attempt to distinguish definitely between callus and wound-cork. Frank³ defines wound-cork as being formed by the passage of pre-

¹ Dale, Philos. Trans. Roy Soc., vol. 194, p. 163, 1901.

² loc. cit., p. 61.

³ Frank, loc. cit. pp. 59, 60.

existent cells directly into cork-cells by a renewal of meristematic activity, and wound-callus, on the contrary, as arising by an apical growth in the direction of the wound, taking place in the underlying cells and leading to the formation of unseptate tubes or rows of cells, which form a scar at the wounded surface.

Massart¹ considers this distinction not to be strictly applicable to leaves, as growth—a callus character—is nearly always combined with the regular wound-cork formation.

That there should be a third alternative in the formation of an absciss-layer, determined merely by difference in external conditions, suggests that all these processes are conditional variations of the fundamental reaction to a traumatic stimulus which is directed to breaking the continuity of organization between the dead and the living cells.

¹ Massart, loc. cit., p. 53.

EXPLANATION OF PHOTOGRAPHS IN PLATE XXIX.

Illustrating Dr. Blackman's and Miss Matthaei's paper on the Reaction
of Leaves to Wounds.

The plate gives reproductions of six natural-sized photographs taken from the living leaves.

Figs. 1, 2 are from one leaf showing the collective treatment of dead areas that are close together. Fig. 1 is an early state, but in Fig. 2 the process is nearly complete and the piece is hanging.

Fig. 3 is a late stage in the abscission of circular dead patches. The inner hole in each spot has been punched out and the outer concentric ring of separation is the work of the absciss-layer. The rings thus cut out are mostly still attached at one point.

Figs. 4, 5 are from the same leaf; showing firstly the stage at which the occlusion-line is visible running round each brown area of dead cells, and secondly the final state in which all the pieces have been exfoliated and the new edges have finished growing.

Fig. 6 shows a leaf with two killed areas in the shape of longitudinal strips. This is in an intermediate state between those of the first and second figures and the pieces to be exfoliated are not quite free yet.

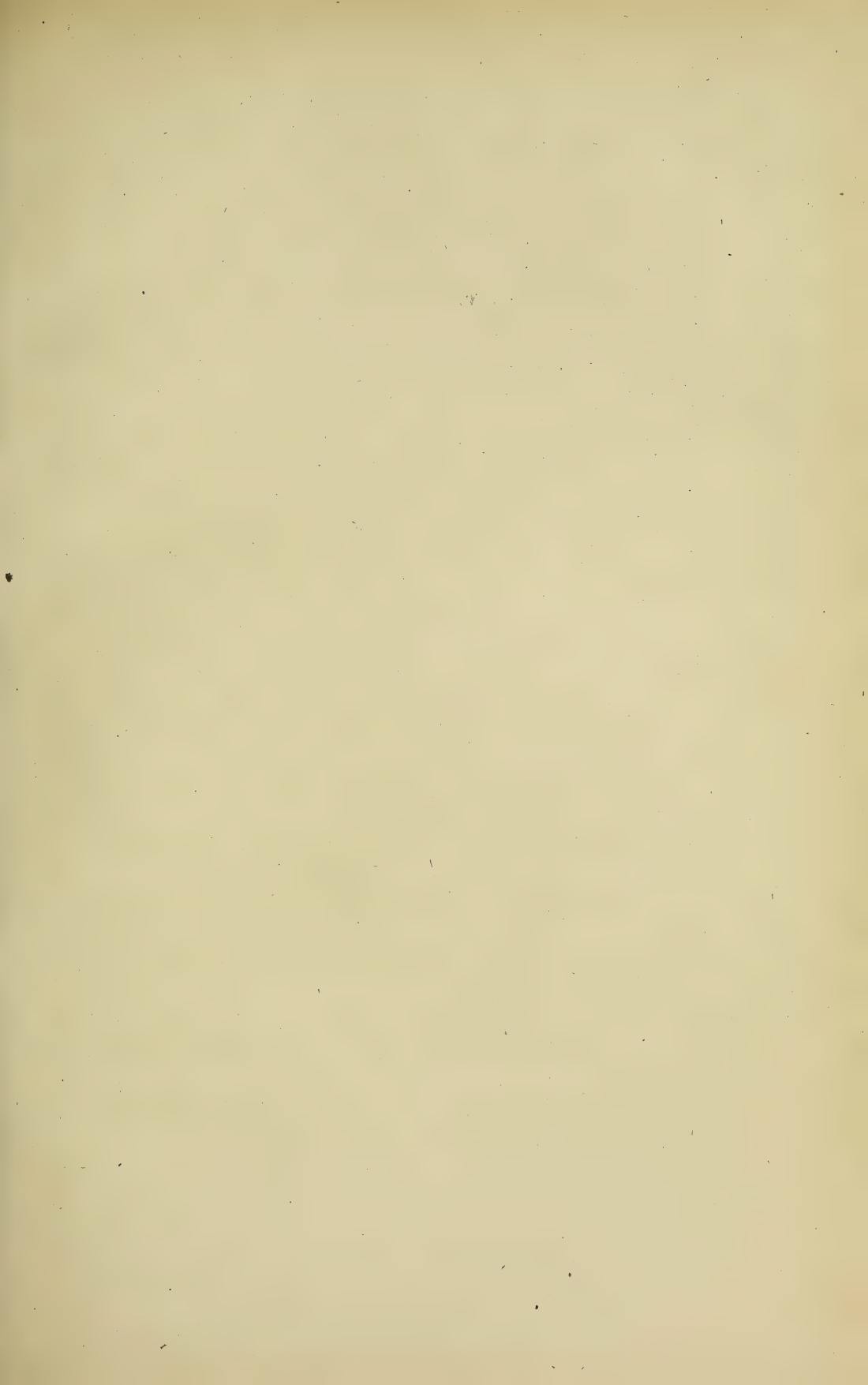




FIG. 1



FIG. 2

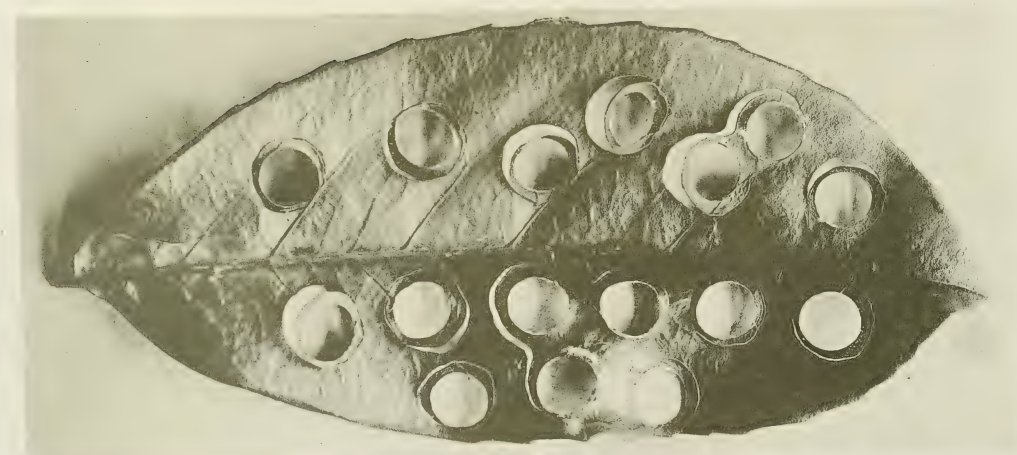


FIG. 3

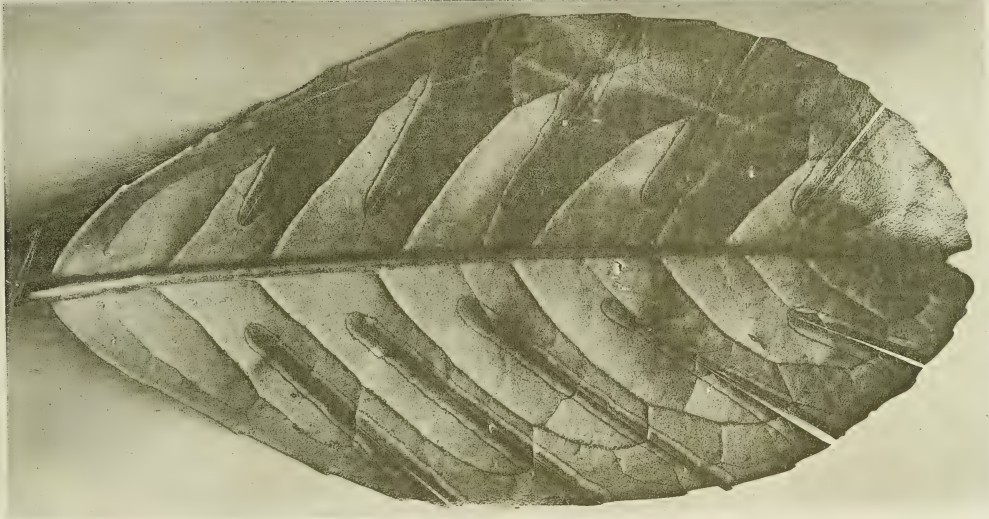


FIG. 4

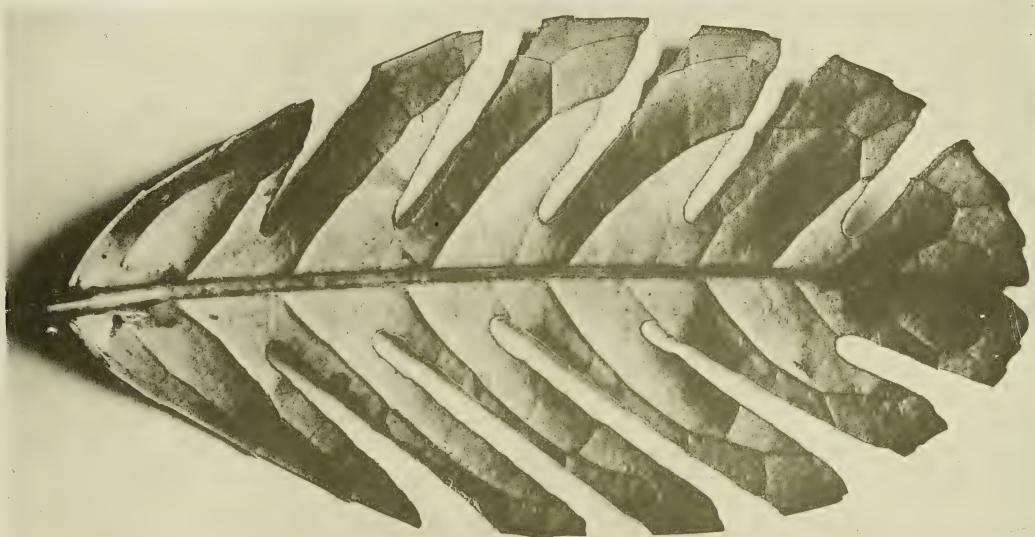


FIG. 5

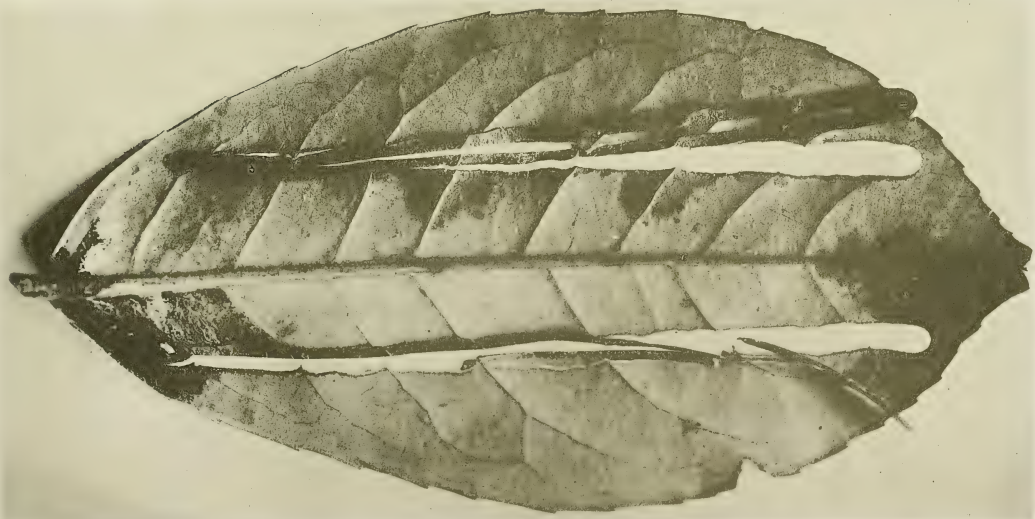


FIG. 6



FIG. 1

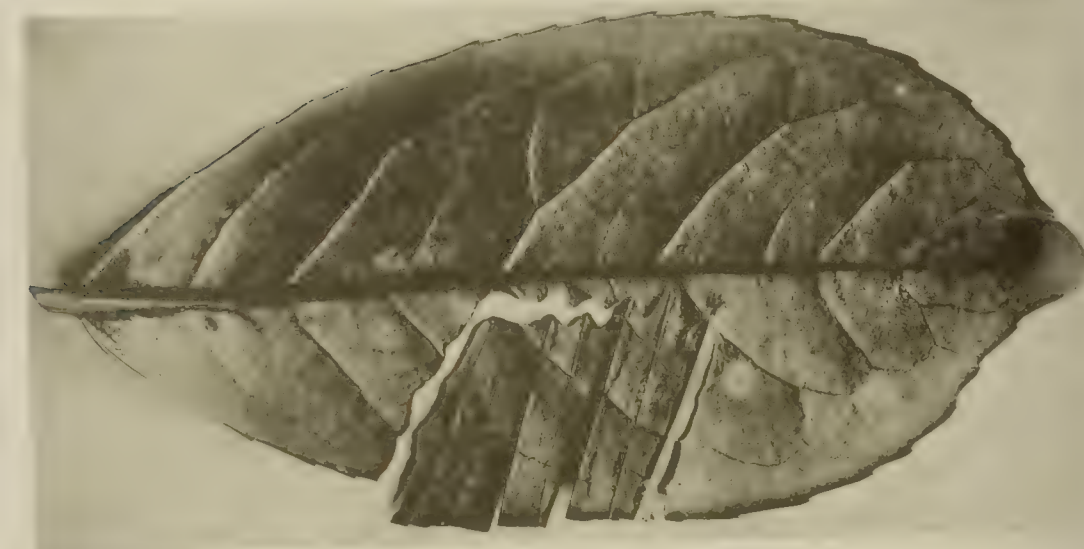


FIG. 2

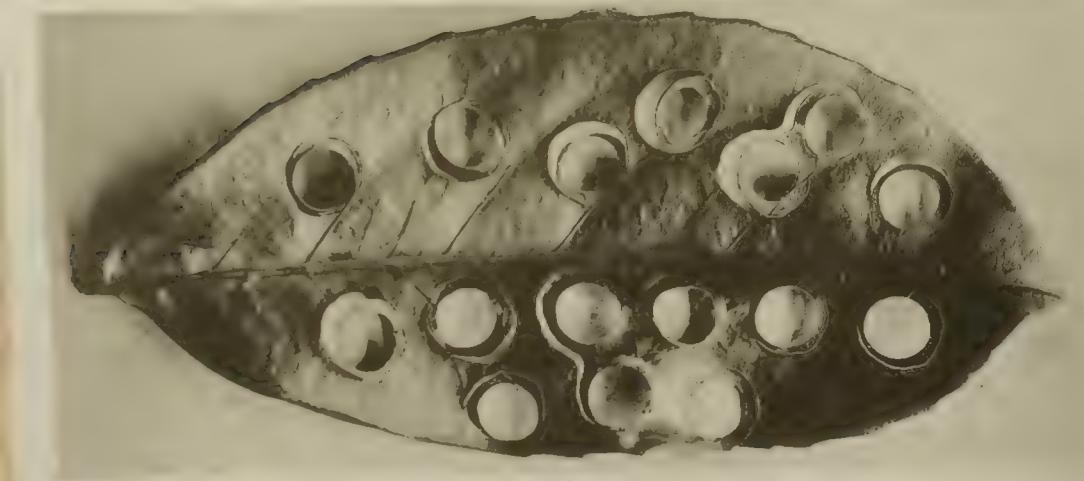


FIG. 3



FIG. 4

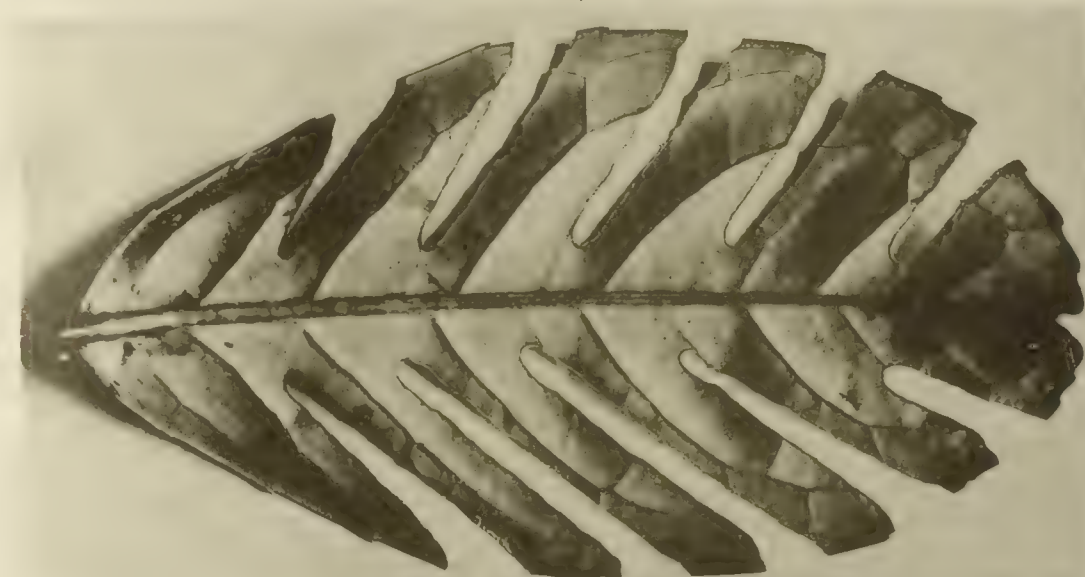


FIG. 5



FIG. 6

Morphological Notes.

BY

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Director, Royal Botanic Gardens, Kew.



With Plate XXX.



II. PERSISTENCE OF LEAF-TRACES IN ARAUCARIEAE.

WHEN I wrote the note in the last number of the *Annals of Botany*, I was under the impression that the persistence of leaf-traces was a wood-structure confined to the species of *Araucaria*. It now proves to be possessed by other genera belonging to the *Araucarieae*.

I had repeatedly examined a large slab of the wood of Kauri Pine (*Agathis australis*) in the timber museum at Kew without detecting any trace of the structure. I was therefore surprised to notice it as conspicuous as in *Araucaria* in planks of Kauri Pine which I recently examined in the Botanical Institute of the University of Glasgow. I now find it equally so in a hand specimen of the same wood in our museums presented by the Admiralty. It is scarcely less obvious in a specimen of the wood of *Agathis robusta*.

It is also beautifully conspicuous in the early annual zones in the wood of *Cunninghamia sinensis*. In this the leaf-traces certainly persist for some years, but the dimensions of the specimen do not enable me to say to what extent.

[*Annals of Botany*, Vol. XV. No. LIX. September, 1901.]

I fail altogether to find any trace of it in the only available specimen of the wood of *Sciadopitys verticillata*, a genus which is included by Bentham and Hooker in the tribe *Araucarieae*: As *Sciadopitys* has been removed by Dr. Masters to the *Taxodiaceae*, the persistence of leaf-traces may be asserted to be a characteristic feature of the former group.

III. THE CARPOPHYLL OF ENCEPHALARTOS.

Many years' study of the *Cycadeae*, the species of which from various causes—which I hope some day to elucidate—have been much misunderstood, assures me that important distinctive characters in the sub-tribe *Encephalarteae* are afforded by the female cones. The carpophylls terminate exteriorly in shield-like expansions, rhomboidal in outline, the superficial sculpture of which in most cases affords unmistakable specific differences. This is apparent at a glance if Fig. 1, *Encephalartos villosus*, Lem., Fig. 5, *E. longifolius*, Lehm., and Fig. 6, *E. brachyphyllus*, Lehm., are compared.

I must confess that I had not given much thought to the meaning or origin of these curious structural differences till, some years ago, I had the pleasure of receiving from my friend Herr H. Wendland, Director of the Botanic Garden at Herrenhausen, Hanover, the first example, as far as I know, of a monstrous Cycad cone. The species was *Encephalartos villosus*, Lem. The cone was for the most part normal except in the upper part, in which the carpophylls had become foliaceous. Fig. 1 is a reproduction of a faithful drawing made at the time by Lady Thiselton-Dyer.

In *Cycas* itself, the morphology of the carpophylls presents no difficulty. They alternate on the main axis with foliage leaves, and are their precise equivalents. But in the *Encephalarteae* all trace of foliar structure in the carpophylls is apparently lost, and I must confess that, though obvious enough when one sees it, I should never have arrived at their correct interpretation without the help of Herr Wendland's striking specimen.

Taking such a case as Fig. 6, *E. brachyphyllus* one might

look a long time without seeing the foliar homology. The peltate extremity of the carpophyll offers a flat expansion, hollowed out in the centre by a shallow rhomboidal excavation. It is only by a comparative study that the meaning and origin of this becomes intelligible.

In Fig. 1 it will be observed that one of the carpophylls has been replaced by, or, if you like, has grown out into a leaf, *a*, reduced in size yet presenting all the essential characters of a normal leaf of the species. At *b* other carpophylls have grown out and taken a foliaceous habit: these are, however, so generalized and reduced that without the help of the more fully developed leaf, their equivalence would be scarcely intelligible. This much is clear: the solid expanded peltate carpophyll is nothing more than a transformed foliage leaf, and capable of being replaced by it.

Now to digress a moment. The carpophylls of *E. villosus*—which is the representative of a small section of the genus confined to eastern tropical Africa from Natal northwards—have the upper margin of the rhomboidal peltate surface prolonged and bent over and downwards, terminating in a toothed edge. A study of the drawing of Herr Wendland's interesting specimen must, I think, lead to the conviction that the teeth correspond to the laciniae of the imperfectly foliaceous carpophylls, and so lead up to the fully developed pinnate leaf. The teeth, then, are the ultimate reductions of pinnae.

It will, however, be noticed that the foliage leaf ends abruptly, and that its apex is atrophied. The rhachis is more or less four-angled, and the terminal cicatrix is therefore obtusely rhomboidal. At *c* it will be seen that would-be foliaceous carpophylls have been arrested in their development and have atrophied truncated extremities.

If we now pass to Figs. 2-5, which are copied accurately by Mr. G. T. Gwilliam from a life-sized photograph of the female cone of *E. longifolius*, we can hardly fail to see that the fully developed cicatrix is gradually developed from the atrophied apex of the carpophyll. The figures are taken at successive levels from the top downwards: Figs. 2-4 are sterile carpo-

phylls; Fig. 5 is an ovule-bearing one. The cicatrix has expanded and become the more prominent feature in the sculpturing of the peltate expansion. Its surface is filled up with tubercles, which probably have some relation to the ends of the fibro-vascular bundles.

Encephalartos brachyphyllus is remarkable for the relatively small number of carpophylls which compose its cone. These consequently attain an unusually large size. The terminal cicatrix is expanded into the large shallow depression already referred to, which occupies the greater part of the external surface of the peltate extremity.

As already pointed out in *E. villosus*, the carpophyll is a reduced and modified equivalent of an entire foliage leaf, the pinnae of the lamina being represented by teeth. In *E. longifolius*, *E. brachyphyllus*, and other species, this is not so, and apparently nothing but the petiole or rhachis has contributed to the structure of the carpophyll. In Fig. 5 it will be noticed that the peltate extremity bears usually four more or less well-marked ridges; two lateral and two vertical. These correspond to similar ridges on the foliar rhachis.

In these cases the carpophyll is entirely petiolar. Below, and concealed by the peltate extremity, it bears a pair of ovules, one on either side. The interesting question suggests itself as to what is the homology of these. In *Cycas*, Sachs (Textbook, 2nd ed., p. 503) says 'the lower pinnae are replaced by ovules.' Probably this is the ordinary view which would be taken for *Cycadeae* generally. But an ovule is a sporangial structure, and it is not easy to see anything in a pinna which is in any way comparable to it. Morphological conceptions must not enslave us, and I see no reason why sporangial structures, like buds, may not appear anywhere.



Fig. 4.



Fig. 6.

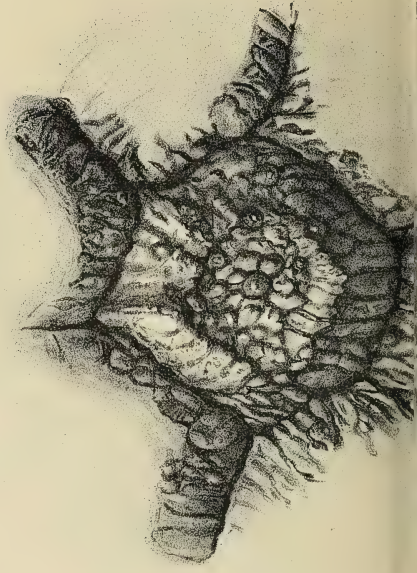
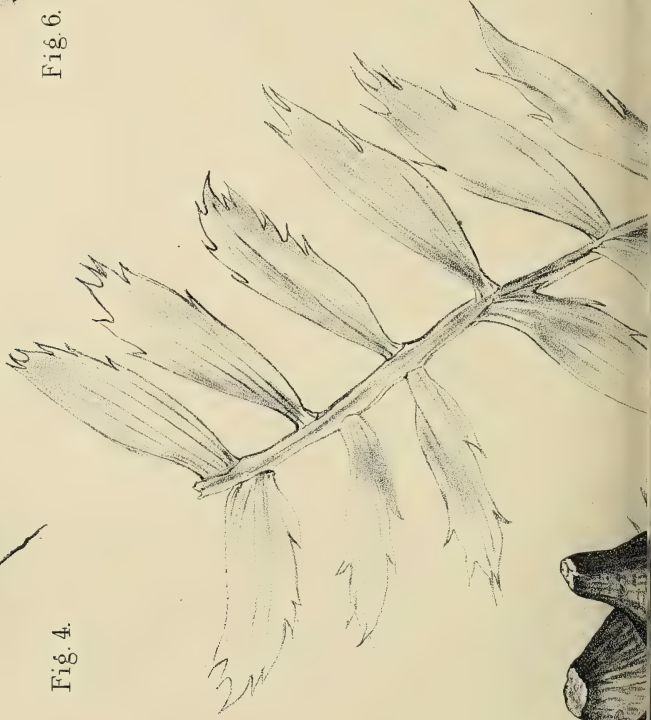




Fig. 5.

Fig. 2.

Fig. 3.

Fig. 1.

DYER. — ENCEPHALARTOS.

H.T.D. del.

University Press, Oxford.



Fig. 4.

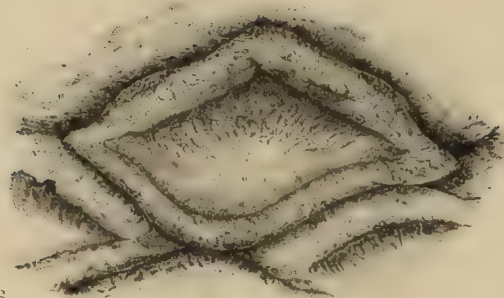


Fig. 6.

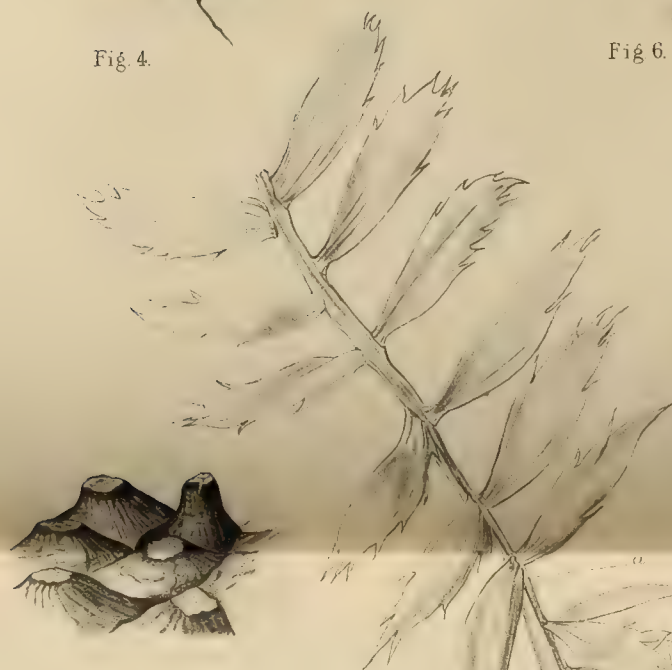


Fig. 2.

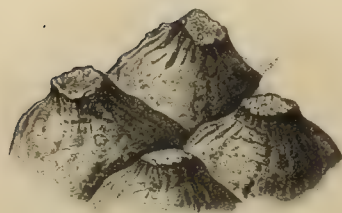


Fig. 3.

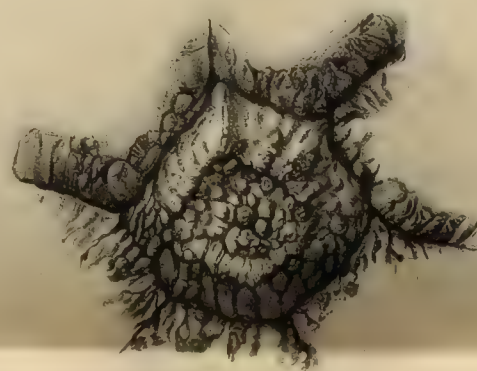


Fig. 5.

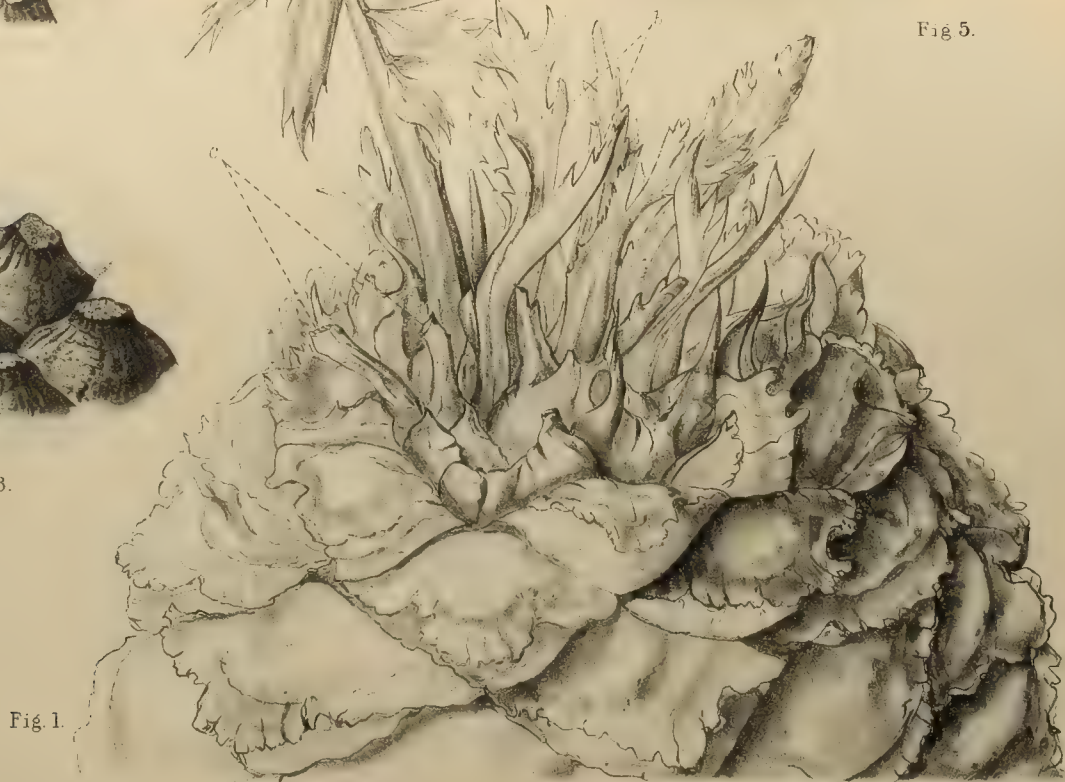


Fig. 1.

NOTES.

THE OPENING OF THE NEW BOTANICAL DEPARTMENT AT THE GLASGOW UNIVERSITY.—On June 13, 1901, as a part of the celebrations on the ninth Jubilee of the University of Glasgow, the new Botanical Department was formally opened by Sir Joseph Hooker. The building has been erected after the design of Mr. J. O. Scott and Mr. J. Burnet, and has cost over £17,000. It includes a lecture-room to hold 250, elementary laboratory to seat 100, advanced laboratory, museum, herbarium, private rooms, dark rooms, &c.

Principal Story, in a speech acknowledging the benefactions from which the cost of the building had been met, referred to the hereditary connexion of Sir Joseph Hooker with Glasgow, his father having been for twenty years the Professor of Botany in that University, of which he himself is a graduate.

Sir Joseph Hooker's opening address was as follows:—

Ladies and Gentlemen,

My friend Professor Bower has done me the honour of asking me to perform the function of declaring these buildings open, and to preface it by giving my earliest recollections of the botanical teacher and his teaching in this University. To do this I must ask you to imagine yourselves carried back to the first quarter of the last century, a time so long gone by, that what I have to relate must be regarded rather as impressions retained than memories recalled.

My father was then the Professor of Botany, and to understand his position at the commencement of his career as such, I must give you an outline of his previous life as a botanist. He had not been educated for the medical, or indeed for any other learned profession. Having inherited ample means, and having been from childhood devoted to the study and collection of objects of natural history, he

determined to devote his life and his fortune to travel and scientific pursuits. Beginning with ornithology and entomology, he subsequently confined his attention to botany, and a few years after coming of age undertook a voyage to Iceland, where he made large collections with notes and observations. These were totally lost through the rapid destruction by fire, in mid ocean, of the ship in which he was making the return voyage to England. His life and those of the crew were saved through the fortuitous arrival of a vessel that should have left Iceland before them, but which had been providentially detained. On his return, he printed for private distribution his 'Journal of a Tour in Iceland,' which was shortly afterwards published in two volumes. This secured his election as a Fellow of the Royal Society at the early age of twenty-seven. Having been frustrated in his intention to visit Ceylon (for which he had completed his outfit) through disturbances in the island, and from going to Brazil or South Africa by the war with France, he turned his attention to Scotland, where he made three extended botanical tours, travelling in the Highlands on foot, on horseback, or by boat, as far as the Orkneys and Skye. He also spent nine months botanizing in France, Switzerland, and the North of Italy. In the intervals he devoted himself to the study of Cryptogamic plants, chiefly Mosses and Hepaticae, upon which Orders he published works that established his reputation as a systematic botanist.

Early in 1820, reduced circumstances requiring him to turn his botanical attainments to material account, he obtained, through the influence of his friend Sir Joseph Banks with George III, the chair of Regius Professor of Botany in this University. It was a bold venture for him to undertake so responsible an office, for he had never lectured, or even attended a course of lectures; and in Glasgow, as in all other Universities in the kingdom, the botanical chair was, and had always been, held by a graduate in medicine. Owing to these disqualifications his appointment was naturally unfavourably viewed by the medical faculty of the University. But he had resources that enabled him to overcome all obstacles: familiarity with his subject, devotion to its study, energy, eloquence, a commanding presence with urbanity of manners, and above all the art of making the student love the science he taught. Success attended the delivery of his first course, hastily as it had to be prepared, as was proved by his students presenting him, at the close of the course, with

a handsome silver vasculum chased with the design of a moss that had been named after him, in token of their appreciation of his lectures. So, too, at the end of his second, he received a similar recognition, in the shape of a beautifully bound early edition of Scott's poetical works in ten volumes.

But his energies were not confined to lecturing: feeling the want of a manual on the Scottish Flora to put into the students' hands, he published in time for use in his second course the *Flora Scotica* in two volumes, the outcome mainly of his earlier Scottish expeditions; and in readiness for his third course he produced at his own cost, and from drawings made by himself, an oblong folio of twenty-one lithographed plates, with descriptions of the organs, &c. of upwards of three hundred plants. A copy of this work was placed before every two students in the class during that portion of each day's lecture that was devoted to the analysis of plants obtained from the garden and placed in the students' hands for this purpose. I should mention that every student was expected to provide himself with a pocket-lens, knife, and pair of forceps, aided with which they followed the demonstrations of the Professor. I think it may fairly be said that these early lectures heralded the dawn of scientific botanical teaching in this University.

Another claim upon the Professor's energies was due to the fact that the botanical class was in a great measure ancillary to that of *materia medica*, a practical knowledge of which latter subject was at that time required of all candidates for a medical degree, diploma, or licence, by, I believe, all the examining bodies of the United Kingdom. Now the Glasgow students of botany were, with few exceptions, preparing themselves for the medical profession, and a considerable portion of them at that time looked forward to service in the army, navy, India and the Colonies, where they would be thrown on their own resources for ascertaining the quality of their drugs, which had either undergone a long voyage from England, or had to be replaced by such substitutes as the practitioner's knowledge of botany might enable him to discover. The Professor hence devoted much time to teaching the botanical characters of the principal medical and economic plants. To this end he made large coloured drawings of them in flower, fruit, &c., which were hung in the classroom when the Natural Orders to which they belonged were being demonstrated, and he passed round dried native specimens of them

taken from his herbarium, or living ones from the garden when they were to be had, together with samples of the drugs or other products which they yielded. I may add that this collection, which he presented to Kew, was the germ of the three museums of economic botany, the first ever established, that are such important features of the Royal Gardens.

It remains to allude to the class excursions, which have always been, and still happily are, a prominent feature of the botanical teaching in the Scottish Universities. Of these there were three: two, on Saturdays, were habitually to Campsie Glen and Bowling Bay respectively. The third, which was eagerly looked forward to by the most ardent of the students, took place at the end of June, during what was called the 'preaching week,' when the lecture-room was closed. It was to some good botanizing ground in the western Highlands. As many as thirty students have taken part in one of these larger excursions, each provided with as small a kit as possible—a vasculum, and apparatus for drying plants. They were often accompanied by students from Edinburgh, and sometimes by eminent botanists, British and foreign. In those days there were few inns in the western Highlands, and fewer coaches, and the roads were bad. On one of my father's first excursions to some mountains beyond the head of Loch Lomond he provided a marquee holding thirty persons, which was transported in a Dutch waggon by a Highland pony; and for supplies the party depended upon the flocks and fowls of the cottagers. On the first upon which I was taken as a boy, to Ben Lomond, there was no inn at Tarbet, and we all slept there in our clothes, on heather spread on the floor of a cottage. On another occasion when I was allowed to join the party (more for fishing than for botanizing) on an excursion to Killin, we walked the whole way from the head of Loch Lomond along the old military road made in the previous century by General Wade, eulogized in the well-known distich:

'If you'd seen these roads before they were made,
You'd have lift up your hands and blessed General Wade.'

If I were asked what I regarded as of most importance to the student in the manner of my father's teaching as sketched above, I would answer that it taught the art of exact observation and reasoning therefrom—a schooling of inestimable value for the medical man, and one that is given in no other profession, but which ought to come,

in this country as it does in Germany, early in the education of every child. I have met many of my father's pupils abroad, in India and the Colonies, who have told me that the botanical lectures gave them the first ideas they had ever entertained of there being a natural classification of the members of the vegetable kingdom. Then with regard to the results in a botanical point of view, the magnetism of the lecturer, and the interest of the subject, imbued many of his pupils with a love of science that proved permanent and fruitful. They made observations and collections for their quondam Professor in the temperate and tropical climates of both hemispheres, some of them throughout their lives, which have very largely contributed to a knowledge of the Flora and vegetable resources of the globe.

After twenty years of professorship my father retired, and undertook the directorship of the Royal Gardens, Kew. Since that period great changes have been introduced in the method of botanical teaching in all our Universities, due on the one hand to a vastly advanced comprehension of the structure of plants and of the functions of their organs, and on the other to a recognition of the fact that the study of the animal and vegetable kingdoms cannot be considered apart. Furthermore, chemistry, physics, and greatly improved microscopes are now necessary for the elucidation of the elementary problems of plant-life. The instruction in these two sciences (chemistry and physics) has, with all others, advanced in this University *pari passu* with that of botany, and kept it in the forefront of the educational establishments of the kingdom. The addition of the building in which we are assembled is evidence of the resolve that it shall not relax its efforts to maintain its well-earned position; and with the conviction that the Botanical Laboratory will prove an invaluable aid to research under the aegis of its distinguished Director, I now, under his authority, declare it open.

Sir Wm. Thiselton-Dyer, who also addressed the company, said this was a very remarkable occasion. He was not speaking merely of the opening of this splendid building, but of the memories which had been recalled in the celebration of the ninth jubilee of the University. There was one idea which had dominated the proceedings—namely, how great was the responsibility which fell on every one in an academic institution, who had to do with education. Lord Kelvin, in an eloquent speech made the previous night, told them that the past, the present, and the future were all one, that the present was the

heir of the past, and that the present was fraught with the responsibility of that which is to come. Now there were circumstances connected with the study of botany in this institution, which had been dwelt upon by his own distinguished predecessor in the institution over which he himself presided, which illustrated the never-ending responsibility of academic work; for if it had not been for the work of Sir Joseph Hooker's father in this University he did not think that Kew would be to-day what it is; and as the University of Glasgow looked to Bologna, so did Kew look to Glasgow. At the commencement of the reign of our late revered Sovereign it had been determined to make of the royal demesne a great scientific institution, and the Government turned to Glasgow and found in Sir Wm. Hooker a man who could administer and develop it. The reason they turned to Sir Wm. Hooker was because he had founded in this University a new and original school; and he carried the traditions of his work from Glasgow to Kew, where they had there been perpetuated. He was told that there were those who thought that this was too large a building to devote to so small a subject as botany. But what subject of interest did botany not touch? Darwin said: 'I love plants; they present all the secrets of life in their simplest form.' They found revealed in plants, in simpler form than could be observed in the animal economy, the inscrutable problem of life, and it was the study of these problems that would be pursued in this building. They had with them Lord Lister, and they knew the service he had rendered to humanity by curbing the lethal ends of obscure branches of the vegetable kingdom in their inroads on suffering humanity. In this great city, with its extensive commerce, there were problems absolutely immeasurable in the investigation of vegetable materials and in their application in the arts. It had been said that even the science of jurisprudence might be approached by the study of botany. The practical study of that science brought the student into immediate connexion with nature, and gave him the power of making accurate observations and drawing conclusions from what he had seen. Sir William concluded by congratulating Professor Bower and the University of Glasgow on the work he had done in connexion with the botanical department.

Lord Lister said it was a very great privilege to propose a vote of thanks to the veteran botanist of world-wide reputation—Sir Joseph Hooker. They rejoiced that the natural physical vigour which he exhibited so remarkably in his most wonderful and fruitful explora-

tions in the Himalayas still remained with him in his advanced years, and enabled him to do what he had done that day. He was sure they all hoped that Sir Joseph Hooker would long remain among them the principal pillar and ornament of botanical science in the world. They most cordially thanked him for the most interesting account he had given of his father's life and labours. It was certainly remarkable that one who began as he did should have been so exceedingly successful as a teacher. His Lordship had heard only yesterday from one of those present at this splendid jubilee that the attendance of students at Sir William Hooker's classes in this University was something phenomenal. He might, perhaps, be permitted, as one who in a very humble way loved botany—loved it dearly—to echo what had been expressed by Sir Joseph Hooker, and hope that the University of Glasgow and all Universities would never allow botany to be left out in the curriculum of medical study. He felt that the study of botany, when taught as it should be, introduced the young student in the most beautiful way to the study of biology, and it cultivated those habits of independent and accurate observation which were of such enormous importance, and which were apt to pass uncultivated in later medical study. Then, to echo what had been said by Sir Joseph Hooker and Sir William Thiselton-Dyer, one knew more and more that all life, whether animal or vegetable, was, so to speak, one; and with the great advance that had been made in late years in the knowledge of the vegetable kingdom, there could be observed and demonstrated with the utmost readiness many of the most important physiological truths and principles which applied to animals. It surely could not be a matter altogether of indifference to a medical man that he should be absolutely ignorant of the source of the vegetable articles of the *materia medica*. There might have been excess of attention devoted to this branch of medical study, but to neglect it absolutely seemed to him to be the most profound error. There were other subsidiary advantages from botanical study. If a man had not studied botany, how could he accept the post of analyst of an exploring expedition; how could he avail himself of the advantages he might have if he were placed as a medical man in some distant part of the world where there were objects all around him—vegetable as well as animal—well deserving his investigation and study? Lastly, he knew from experience that botany gave lifelong delight to the country. There were those who, when they went to the country, must have the excitement of sport

to give them interest in it; but the botanist, whether he made his rambles at home or abroad, was always sure of making new acquaintances in the vegetable kingdom or recognizing dearly-beloved old acquaintances. Therefore, from these various points of view, he ventured to express the earnest hope that botany would always be taught to the medical students of Glasgow University. Lord Lister concluded by proposing a vote of thanks to Sir Joseph Hooker for his address.

Professor Bayley Balfour seconded the motion. As a former Professor of Botany in this University, one who never dreamed of or aspired to these splendid halls for teaching botany, he warmly congratulated Professor Bower on having the energy, perseverance, and persuasiveness by which he had managed to secure so magnificent an equipment. It was most fitting that these halls should have been opened by one whose teaching had dominated botanical science for the past half-century, whose investigations had ranged over every field of botanical work, and whose influence upon the scientific thought of this country as a co-worker with Darwin was altogether immeasurable. Allusion had been made to the long connexion of the Hookers with Glasgow University. It was remarkable that a connexion, first formed so long ago as 1820, should at the beginning of this new century be strengthened by the opening of these halls by Sir Joseph Hooker. Under the imprimatur of no higher name in the world of science could Professor Bower enter on the work he had to do in these halls.

The proceedings then terminated.

SIMPLE APPARATUS FOR THE MEASUREMENT OF TRANSPIRATION FROM A SHOOT.—This modification of a well-known apparatus for the demonstration of transpiration from a shoot will be found useful in a laboratory, as it admits of a series of quantitative measurements in a short time and is easy to set up and adjust.

The bottle *A* is provided with a good rubber stopper pierced with three holes, one about 12 mm. in diameter, the other two about 5 mm. each; through one of the smaller holes the shoot is pushed, the other carries the gauge *B*, made of capillary tubing about 60 cm. long. Through the third larger hole passes a rod of wood terminating in a knob *C*; this serves to adjust the water to the zero position in

ERRATA.

Page 558, line 10. *For 'never' read 'had'*

„ „ *For 'or' read 'and'*

the gauge, as by forcing the rod into the bottle the water is driven out along the capillary tube.

As a preliminary, two marks are made on the gauge tube at a known distance apart; the capillary tube is also calibrated by filling with mercury and weighing; in the example figured the distance between the marks is 50 cm., and 1 cm. of the capillary tube contains .00895 c.c.

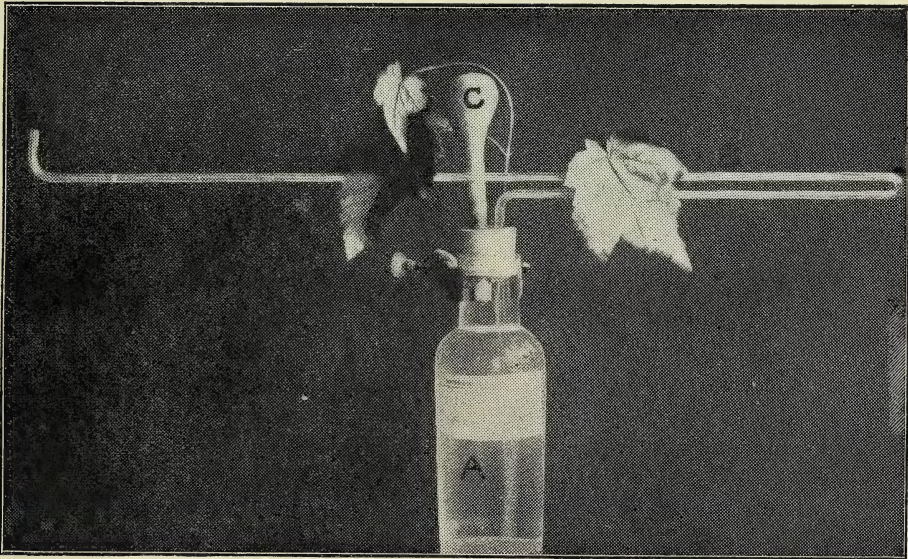


Fig. 9.

The stem of the shoot selected for experiment is pushed through the hole in the stopper, the bottle filled with distilled water and the stopper inserted, excluding any air-bubbles; after a few minutes the rod is gently screwed into the bottle till the water in the capillary tube is forced past the mark. The water begins to recede in the tube as it is absorbed by the shoot; the time is noted that elapses as the water recedes from the first to the second mark. The water can then be forced back to the zero and a new determination made under varying conditions of temperature, illumination, &c. Finally, the area of the leaves can be ascertained by tracing them on to squared paper.

Examples:

(1) Bright day—temp. = 20°C . Sycamore shoot.

In window—air still.

Time required = 39 min.

Water transpired = $50 \times .00895 = 0.447$ c.c.

Area of leaf = 155 sq. cm.

1 sq. metre of leaf transpires 44 c.c. per hour.

(2) Bright diffuse daylight—temp. = 21°C .

Out of doors.

Time required = 16 min.

Water transpired = 0.447 c.c.

Area of leaf = 286 sq. cm.

1 sq. metre of leaf transpires 59 c.c. per hour.

A. D. HALL.

SOUTH EASTERN AGRICULTURAL COLLEGE, WYE.

THE BROMES AND THEIR RUST-FUNGUS (*Puccinia dispersa*).—I have for some time been occupied with an investigation which has involved a comparative examination of the 'seeds' and seedlings of all the species and varieties of the genus *Bromus* that I could obtain, and a study of the conditions of infection of these grasses by the Uredo-form of *Puccinia dispersa*, the Rust-fungus so common on certain species of them.

The details of the results will, I hope, appear in due course, but some points of interest may be summarized now.

The uredo-spores germinate at all temperatures between about $10-12^{\circ}\text{C}$. and $25-27.5^{\circ}\text{C}$., which may be considered the minimum and maximum cardinal points; the optimum is about $18-20^{\circ}\text{C}$., and many failures in infection were found to be due to the fact that the leaves of the grass may be at temperatures above the maximum. Uredo-spores in water frozen for ten minutes into solid ice germinated on thawing, but freezing for two hours seems to kill them.

Infection experiments show that spores originating on a given species—e.g. *B. mollis*—easily infect that species, but not necessarily another species, even if closely allied, and indeed may not infect all varieties of that species. Thus spores from *B. sterilis* easily infect *B. sterilis* and the closely allied *B. madrilensis*, but not *B. mollis* or its allies *B. secalinus*, *B. arvensis*, &c., and not even the more closely

allied *B. maximus*. Broadly speaking, the uredo-spores, growing on a species belonging to the *Serrafalcus* group, infect other members of that group—in varying degree, however, and not necessarily all—but fail to induce the development of pustules on species belonging to another group—e.g. *Stenobromus* or *Festucoïdes*.

There is evidence, however, going to show that an occasional adaptation to another species may occur in cases where infection does not usually succeed. In such cases, the Uredo having once established itself on another species, its uredo-spore progeny will thenceforth readily infect that species.

The rule seems to be that the uredo-spores infect most easily the species and variety on which they have been developed, less easily varieties or species more remote, and fail altogether to gain a hold on more distant ones.

This does not appear to be a matter depending merely on the structure of the leaves of the host plant, or on any recognizable excretion from them; at any rate the microscope shows none such, and experiments with *B. mollis* and *B. sterilis* demonstrated that the germ-tubes grow readily in filtered extracts, boiled or unboiled, of the leaves. The matter evidently depends on the influence of the previous nutrition of the Fungus, as well as on the reactions of the species attacked, and presents problems of great complexity.

It is possible to grow pure cultures of the grass as well as of the Fungus for weeks and even months in closed and in aerated tubes. 'Seeds,' sterilized by various reagents, have been germinated in large tubes, on cotton-wool supplied with pure nutritive materials, and excellent plants thus raised out of contact with any but filtered air. Such pure cultures have been infected with uredo-spores, and the progeny utilized for similar re-infections, thus ensuring pure cultures of both host and parasite.

'Seeds,' from infected plants, thus treated do not give rise to infected plants; and unless spores are sown on the leaves of the latter no pustules are developed. Moreover, pustules only arise in these cases on the spots where the spores were sown, and within the usual incubation period. These facts seem to militate against any theory of internal seminal sources of disease. It is hoped that by longer series of such pure cultures more definite information on several obscure points will be obtained.

At present the evidence points to the following conclusions. The

acts of infection and incubation occupy about ten days, and many exigencies may prevent the germination of the spores, the entry of the germ-tubes into the stomata or the successful growth of the mycelium in the tissues.

Experiments go to show that the lack of certain minerals—e.g. potassium or phosphates—cause a starvation of the Fungus; partial etiolation of the host, or any other hindrance to free nutrition, assimilation, transpiration, &c., also act detrimentally to the well-being of the mycelium.

Every degree of virulence of attack seems to be shown by the spores in their germ-tubes, either on different varieties or species of host, or on the same host in different conditions.

It appears that in certain cases the germ-tubes may kill the tissues, and cause them to turn black and shrivel up as if corroded. In such cases the parasite can make no further progress, and infection fails. In other cases a mycelium is developed, but lies dormant and produces no spores; we may compare the Fungus in this case to a plant in poor soil, or suffering from drought, &c.

Seedlings with 'spears' only a millimetre or two high can be infected by applying spores to the 'dew-drops' issuing from the water-stomata at the apex of the first leaf.

H. MARSHALL WARD.

CAMBRIDGE,
July 27, 1901.

The Proteolytic Enzyme of *Nepenthes* (III).

BY

S. H. VINES, M.A., D.Sc., F.R.S.

Sherardian Professor of Botany in the University of Oxford.

THE occasion of my reversion to this subject is the publication of researches by the late Georges Clautriau (2) which were concluded but a short time before his lamented decease. The paper is of such importance, and is moreover so relatively inaccessible to English readers, that it will not be out of place if I give a short account of its contents.

Clautriau was, I believe, the first to investigate the physiology of the pitchers of *Nepenthes* in the native habitat of the plant. The species which he so studied was *N. melamphora*, growing at a height of 1500–2200 metres near the laboratory which has been erected at Tjibodas on Mount Gedeh, a volcano in the island of Java. The proteid material used in his digestion-experiments was a 10 per cent. solution of egg-albumin rendered incoagulable on boiling by the addition of a small quantity of ferrous sulphate. In consequence of the altitude at which the experiments were performed, the temperature never exceeded 28° C.: digestion was therefore slow, and the experiments prolonged.

The liquid in the unstimulated pitcher was found to be colourless, tasteless, almost odourless, and slightly viscid. On stimulation of the pitcher, the liquid became acid; and when digestion had taken place, it acquired an odour resembling

that of honey, and often an amber colour which Clautriau attributes to tannoid substances derived from the tissue of the pitcher, rather than to any chromogen such as is formed in tryptic digestion along with amido-acids.

The acidity of the liquid was the subject of special attention. The reaction of the liquid had been found by previous observers to be sometimes acid and sometimes neutral: even in unopened pitchers it is often acid, as I have pointed out (11.a, p. 575), a fact which is not readily accounted for. Clautriau made the interesting observation that acidity is caused, not only by the introduction of any foreign body, but also by mechanical stimulation of the pitcher, whether open or unopened. It suffices to shake a pitcher vigorously to induce acidity of the contained liquid in the course of a few hours.

Incidentally attention is drawn to the remarkable fact, well known in other pitcher-plants such as *Sarracenia*, that living insect-larvae, more especially those of the mosquito, are to be found in the pitchers. This fact, Clautriau rightly argues, cannot be accepted as evidence against the digestive activity of the pitcher-liquid: it is rather to be taken as an indication of special adaptation of the larvae. It is a fact belonging to the same physiological category as the presence of living parasites in the digestive tract of animals, and as the indigestibility of the gastric mucous membrane by its own secretions. It is, however, clear that the pitcher-liquid can contain no actively toxic or anaesthetic substances. Clautriau observed, in fact, that insects are very slowly killed when immersed in the liquid.

With regard to the actual process of digestion, Clautriau found that the albumin which he introduced into vigorous pitchers entirely disappeared within two days. The examination of the liquid at this stage showed that it gave no precipitate on neutralization, or on boiling in the presence of acids or of neutral salts: nor was any precipitate caused by ferrocyanide of potassium and acetic acid, by double iodide of mercury and potassium, or by phosphomolybdic acid.

Hence it would appear that the liquid contained no proteid matter of any kind. The conclusion at which Clautriau arrives is that the introduced albumin is rapidly attacked, and that the products of digestion are absorbed as quickly as they are formed. The proteolytic action is, moreover, exerted by an enzyme secreted by the plant, and is not attributable, as Dubois and Tischutkin suggested, to microbes. A further observation of special importance is that when a pitcher is separated from the plant, digestion is at once arrested.

In order, if possible, to trace the various stages in the proteolytic process, Clautriau instituted a series of experiments *in vitro*, the tubes being placed by the side of the plants in the open. In all cases but one, the albumin in the tubes underwent no change: in the exceptional case, a quantity of albumose, with perhaps some peptone, was formed. This negative result he attributes, with considerable probability, to the relatively low temperature. Nevertheless the experiments are of interest, in that they suggest some specific influence of the pitcher upon the digestive process.

On his return to Europe, Clautriau pursued his researches on plants (mostly *N. Mastersiana*) grown in hot-houses. As regards digestion in the pitcher itself, he found, as in his previous experiments in Java, that although the albumin which he introduced disappeared, he could, as a rule, find no proteids in the resulting liquid. For example: 15 c.c. of a watery solution containing 2.5 c.c. of the incoagulable albumin were poured into a pitcher: four days afterwards the liquid was found to have been partially absorbed, the remainder being viscid and amber-coloured, as is usually the case after digestion: but it contained no albumin, syntonin, albumose, or peptone. However in two such experiments he was able to detect the presence of peptone.

These experiments were supplemented by others *in vitro*, of which the following is a detailed example: 3 c.c. of filtered pitcher-liquid were placed in each of three tubes A, B, C, and to each 20 drops of a solution of incoagulable albumin were added: to B was also added one drop of

a dilute solution of hydrochloric acid (amounting to 0.01 c.c. HCl), and the same addition was made to C after it had been heated for about ten minutes in a water-bath at 100° C.: the three tubes were set to digest for three days at 37° C., camphor being used as an antiseptic. The examination of the contents of the tubes then showed that A contained no albumin or syntonin, and only doubtful traces of albumoses, whence it was concluded that here peptonification had been complete: the same result was obtained with B: whilst in C there was no albumin, but a great deal of syntonin, a small quantity of albumoses, and no peptone, results which are entirely attributable to the action of the introduced acid. Commenting on these facts, Clautriau makes the remark that the addition of HCl to the pitcher-liquid, which has been usual in experiments of this kind, is clearly unnecessary, since peptonification took place in the tube A without added acid.

In other similar experiments at a lower temperature (about 20° C.) he found that digestion proceeded very much more slowly. He then raises the question as to whether or not it is actually the case that, as asserted by von Gorup-Besanez (3) and others, the liquid of young unopened pitchers is quite as active as that of open pitchers, provided that it be acidified with HCl. He is inclined to take the opposite view, having on two occasions failed to obtain peptonification with liquid from unopened pitchers.

To the important question as to the nature of the proteolytic enzyme of the pitcher-liquid, Clautriau gives the answer that it is a pepsin: that is, an enzyme acting on the higher proteids in an acid medium, giving rise to peptones, but incapable of decomposing proteids into non-proteid substances such as leucin and tyrosin. He briefly criticizes the view which I have expressed (11 *b*, p. 555) that the enzyme is not peptic, but tryptic, in its action.

In conclusion, I would briefly mention his interesting observations on the absorption of the products of digestion in the pitchers. Using a solution of methylene-blue, he found that on introducing it into a living pitcher, the colouring-matter

readily penetrated into the glands whilst the epidermal cells stained but slowly. This differentiation was still more marked when methylene-blue was introduced into the pitcher together with some albumin, in which case the colouration was seen to extend into the tissues beyond the glands. At the same time the cytoplasm of the glands showed marked aggregation. It appears, therefore, that the glands not only secrete the digestive fluid, but are also the agents in absorption.

I should have been glad had it been possible for me to do no more than to express my appreciation of so much valuable work in a subject which has long interested me, to confirm the accuracy of the observations, and to recognize the validity of the conclusions. There are, however, certain points connected with Clautriau's experiments *in vitro* upon which I am reluctantly compelled to join issue.

In the first place, I take exception to his inference that the addition of HCl to the pitcher-liquid, as practised by other observers, is useless. It is perfectly true that if the pitcher-liquid be naturally acid, it will digest proteid without any addition of HCl. I have frequently found this to be the case: but I have also found that if a small quantity of HCl or an organic acid, such as citric, be added, digestion proceeds very much more rapidly, so that instead of extending over days, as did Clautriau's experiments, a few hours suffice. Moreover, neutral pitcher-liquid will not digest at all. Furthermore, the experiment upon which Clautriau bases this opinion is inconclusive, because no observations were made to determine the relative rate of digestion of the albumin in tubes A and B: had this been done, it is probable that the albumin in the acidified tube B would have been found to disappear some time before that in tube A.

Secondly, with regard to the doubt which Clautriau expresses as to the presence of the enzyme in the liquid of unopened pitchers, my experience entirely confirms the accuracy of the statement made by von Gorup-Besanez that this liquid is very active when properly acidified.

But the really important divergence between Clautriau's

conclusions and my own is as to the nature of the proteolytic enzyme of the pitcher-liquid, as manifested by the products of its activity. I need not attempt any reply to his criticisms beyond the remark that they appear to be based upon an imperfect acquaintance with my papers. The question at issue is simply one of fact: is the enzyme a pepsin or a trypsin?—a question of some moment, for if it be a pepsin it will be the first instance of such an enzyme having been definitely proved to occur in plants. We agree that the enzyme produces peptones; but here, Clautriau asserts, its activity ceases; whilst I have endeavoured to prove that it proceeds to the further stage of forming leucin, tyrosin, and other substances characteristic of tryptic digestion. Clautriau's evidence is of a negative character: he states that he has failed to detect leucin or tyrosin, but the investigation made with this particular object in view was by no means exhaustive. On the other hand, I have pointed out (11 *a*, p. 580) that there is to be found among the products of digestion a substance which presents some of the characteristics of leucin, though the presence of tyrosin was not detected.

It may be fairly urged that the evidence which I have adduced is inconclusive; and that the only absolutely convincing proof would be the separation of leucin, and of tyrosin too, in sufficient quantity to admit of ultimate analysis. This proof I am not able to give, and for the reason that I have not, so far, been able to carry on digestion-experiments on a sufficiently large scale. Comparing my results with those of Martin (6) in papain-digestion, and those of Chittenden (1) in bromelin-digestion, I am led to infer that the enzyme of *Nepenthes*, which may be conveniently termed *nepenthin*, produces leucin and tyrosin in smaller quantity than does that (papain or papayotin) of the Papaw (*Carica Papaya*, L.) or that (bromelin) of the Pine-Apple (*Ananas sativus*, Schult.); just as these enzymes, in turn, are less active than animal trypsin. This remark applies especially to tyrosin, which is always produced less abundantly than leucin in tryptic digestion;

hence Martin was unable to find any crystals of tyrosin in his experiments with papaïn, though he ascertained its presence among the products of digestion by Millon's reaction.

Short of the actual separation of these substances, there is no qualitative means of detecting them in the presence of peptones and albumoses which, of course, always occur in digestion-liquids. I have endeavoured to precipitate these proteids by means of absolute alcohol; but the albumoses or peptones formed in nepenthin-digestion are remarkably soluble in alcohol, so that when the residue obtained by evaporating to dryness a digestion-liquid is extracted with absolute alcohol, the solution still gives the biuret reaction, showing that albumoses or peptones have been taken up. Tyrosin, it is true, gives characteristic colour-reactions: but these are also given by peptones and are useless when a mixture has to be dealt with.

If I am not in a position, at present, to establish my contention by such direct evidence as to the separation of leucin and tyrosin in measurable quantity would afford, I can at any rate adduce indirect evidence of a convincing character. As long ago as 1831, it was observed by Tiedemann and Gmelin (10) that on the addition of chlorine-water to the liquid resulting from a pancreatic (tryptic) digestion, after acidification, the liquid acquires a colour varying, according to its concentration, from pink to violet; when concentrated, there is a violet precipitate. This colouration is due to the presence of a substance which, together with leucin, tyrosin, and other bodies, is a product of tryptic, as distinguished from peptic, proteolysis. The substance in question is a chromogen, termed *proteinochromogen* by Stadelmann (9), but better known by the name *tryptophan* given to it by Neumeister (8); and its presence affords a ready means of distinguishing tryptic from peptic digestions.

I have found that the liquid resulting from a somewhat prolonged digestion of fibrin by the pitcher-liquid of *Nepenthes*, in the presence of either hydrochloric or citric acid, gives the

tryptophan-reaction. The following is an example of the experiments :—

A mixture was prepared, consisting of 10 grms. moist fibrin (preserved in dilute glycerin), 50 c.c. of 0.3 per cent. HCl, and 50 c.c. pitcher-liquid (*N. Mastersiana*) : this was placed in the incubator (temp. 38.5° C.) at 4 p.m. : next morning at 10.30 a.m. most of the fibrin was found to have been dissolved : a portion of the liquid was poured off, boiled, and filtered : the filtrate gave a good tryptophan-reaction.

The tryptophan-reaction is not, however, exclusively associated with the action of tryptic enzymes, for tryptophan has been found among the products of the disruption of the proteid molecule effected by boiling proteids with baryta-water, or by bacterial putrefaction. But in the absence of all sign of putrefaction, such as the odour of indol and skatol, in my experiments, it must be concluded that the production of the tryptophan was due to the action of the enzyme, and that nepenthin-digestion is tryptic in its nature.

As might be expected, I have also obtained the tryptophan-reaction in liquids resulting from the digestion of fibrin by both Pine-Apple juice and papain.

In order to further demonstrate the tryptic nature of nepenthin, I have made experiments as to its action on albumoses and peptones, using as the proteid material the preparation which is well known as Witte-peptone ; with the result that the digested liquid gives the tryptophan-reaction. The following are the details of one experiment :—

A mixture was prepared consisting of 1 gm. Witte-peptone, 0.2 gm. citric acid, 10 c.c. dist. water, 40 c.c. Nepenthes-liquid : placed in incubator at noon, temp. 38.5° C. ; next morning the liquid gave marked tryptophan-reaction.

I have also found that Pine-Apple juice and papain, under similar conditions, produce tryptophan from Witte-peptone. All the digestion-experiments have been controlled in various ways ; such as using boiled fibrin, in which case the digestion is very much delayed ; or making blank experiments with boiled *Nepenthes*-liquid, or without any at all.

My results make it apparent that the three enzymes,

nepenthin, bromelin, and papaïn (or papayotin), have essentially the same proteolytic action, which is tryptic: though, as I have already pointed out, they seem to differ in activity, bromelin being the most active, nepenthin the least. There is, however, a further difference between them as regards the media in which they are capable of acting. Nepenthin is only active in an acid liquid, and digests when as much as 0.25 per cent. HCl has been added. On the other hand, bromelin, according to Chittenden, and papaïn, according to Martin, are less active in acid than in neutral liquids; and their action is altogether inhibited when the liquid contains about 0.1 per cent. of free HCl. Furthermore, both bromelin and papaïn can digest in liquids which are alkaline to an extent not exceeding 1 per cent. Na_2CO_3 . Of papaïn-digestion Martin says that it is essentially a neutral one; and in view of Chittenden's results, the same description may be extended to bromelin-digestion. It must, however, be borne in mind that both bromelin and papaïn have to act, in the plants in which they respectively occur, in liquid containing organic acid. There can be no question as to the acidity of the tissues of the Pine-Apple; and the fresh latex of the Papaw, like all other latices (see Molisch, 7), is acid. Moreover, I have found by experiment that papaïn can digest fibrin, to the extent of producing tryptophan, in liquids containing up to 0.5 per cent. of citric acid. In this respect these enzymes resemble animal trypsin, which, though most active in an alkaline liquid (1 per cent. Na_2CO_3), can digest proteid in a liquid which is neutral or even slightly acidified with an organic acid.

Including pepsin in the survey, the proteolytic enzymes under consideration may be classified as follows, according to the reaction of the media in which they act most vigorously, so far as is known:—

- | | |
|------------------------------------|-------------------------|
| a. Active only in acid liquids | { Pepsin,
Nepenthin. |
| b. Most active in neutral liquids | { Bromelin,
Papaïn. |
| c. Most active in alkaline liquids | Trypsin. |

If, however, these enzymes be grouped according to their mode of action, the three vegetable enzymes may be associated with trypsin, since they induce that disruption of the proteid molecule which is marked by the formation of tryptophan and other non-proteid bodies such as leucin and tyrosin: on the same ground they would be distinguished from pepsin. But it must be borne in mind that the chemistry of peptic proteolysis is probably by no means so simple as it has been thought to be. Although it has been generally accepted that pepsin does no more than to hydrolyse the higher proteids into peptones, there have long been physiologists, like Hoppe-Seyler, holding the view that non-proteid crystallizable substances, such as leucin, tyrosin, and others, are formed in prolonged peptic digestion. The most recent researches in this direction, those of Zunz (12) and of Lawrow (5), go to prove that such substances are in fact among the products of peptic digestion, without, however, proving their identity with the non-proteid products of tryptic digestion. Lawrow asserts that he has detected leucin in a prolonged (two months) self-digestion of a pig's stomach: but, on the other hand, he failed to find tyrosin. In no case, so far as I am aware, has tryptophan been found in peptic digestion; and until that has been done, the tryptophan-reaction may be taken as the distinguishing criterion between the action of trypsin and that of pepsin. What exactly the relation between peptic and tryptic digestion may be, cannot be determined until a great deal more is known as to the chemical details of both processes. It is not impossible that they may be found to differ, not in kind, as is now generally assumed, but only in degree: in which case the vegetable enzymes enumerated above would form a series intermediate in properties between pepsin on the one hand and trypsin on the other.

The evidence afforded by the tryptophan-reaction strengthens the suggestion which I have already ventured to make (11 *b*, p. 555), that all known proteolytic enzymes of plants are tryptic, though some of them, such as that of *Drosera*, still await investigation.

This suggestion gains in interest when it is borne in mind that tryptic digestion is of general occurrence in the animal kingdom, and is apparently the sole process in many invertebrates. It is not improbable that it may be expanded into the proposition that tryptic digestion is a property of all living organisms, and that it is the more primitive form of the digestive process.

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- 11 *a*. Vines: The Proteolytic Enzyme of *Nepenthes*; Ann. Bot., vol. xi, 1897.
- 11 *b*. *Ibid.* vol. xii, 1898.
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The Histology of the Sieve-Tubes of *Pinus*.

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With Plates XXXI, XXXII, and XXXIII.



THE sieve-tubes of the Coniferae have been previously examined and described by several botanists; but the conclusions at which they have arrived do not always agree together, and a good deal of uncertainty exists in consequence on questions concerning the development and the character of the means of communication between adjoining sieve-tubes.

It therefore seemed advisable to carefully reinvestigate the sieve-tubes of *Pinus* by means of the methods elaborated by Walter Gardiner¹, which have also recently been used to demonstrate the presence of 'connecting threads' throughout the tissues of some species of *Pinus*².

Before the present results are described, some notice must be taken of the work already published on this subject.

¹ W. Gardiner, Roy. Soc. Proc., vol. lxii, 1897. Camb. Phil. Soc. Proc., vol. ix, pt. viii.

² A. W. Hill, 'The Distribution and Character of "Connecting Threads" in the Tissues of *Pinus sylvestris* and other allied Species' (Part I of Gardiner and Hill on 'The Histology of the Cell-wall'). Phil. Trans. Roy. Soc. B., vol. cxciv, 1901, pp. 83-125.

HISTORICAL.

The first mention of the sieve-tubes of Gymnosperms was made by De Bary¹ in 1877, who described and figured those of *Encephalartos* and *Sequoia*. His observations dealt especially with surface-views, in which he noticed the net-like structure of the rounded sieve-areas, and also saw a delicate sieve-structure in the meshes of the sieve. His figures agree closely with Strasburger's surface-view figures, but he failed to notice a development of callus.

The papers, however, which especially deserve attention are those by Janczewski, Russow, Kienitz-Gerloff, and Strasburger.

Janczewski² in 1881 gave a detailed account of the development of the sieve-plate. He found that the developing sieve-plate is composed of more or less refringent particles alternating with each other. In a more advanced stage the more refringent particles, which are also the larger, are found to have become altered into callus at their free surfaces, whilst in the middle they still retain their primitive character, so that the closing membrane shows paired knobs of callus arranged on either side of it.

The alteration of the refringent particles proceeds rapidly until a cylinder of callus is formed, still showing traces of the original unaltered membrane at its centre; but this too is quickly replaced and a solid callus-cylinder or rod is formed. After this the heads of these cylinders or rods swell up and fuse around the cellulose network (composed of the less refringent particles of the primitive wall), and then finally the callus is completely dissolved, leaving open pores between neighbouring sieve-tubes. Thus Janczewski only saw rods of callus traversing the pit-closing membrane, and was not able to observe anything in the nature of protoplasmic connexion, or of the finer sieve-structure figured by De Bary.

¹ De Bary, *Comp. Anat.* (Eng. ed.), p. 179, Fig. 77.

² Janczewski, *Mém. de la Soc. des Sc. Nat. de Cherbourg*, 1881. *Ann. des Sc. Nat., Bot.*, 6^e sér., tome xiv, 1882.

Russow's paper on the development of sieve-tubes¹ was published shortly after Janczewski's, and shows a great advance in knowledge and methods of observation, but it is most unfortunately marred by a total absence of illustration. The omission to append figures to a paper is a great drawback, especially when it deals with questions of minute histology; and no doubt this accounts for the fact that Professor Russow's remarkable papers have not met with the recognition they deserve.

In the paper under consideration Russow first describes the structure of the cell-wall of Gymnospermous sieve-tubes, and then confirms De Bary's results on the sieve-plate, pointing out that the sieve is divided up into irregular fields, each of which is perforated by three to six little holes of fairly different diameter, usually arranged in a circle (Fig. 1, Pl. XXXI). Russow worked mainly with his 'callus reagent²,' which, while it gives excellent staining with callus, stains the protoplasm also, so that it is very hard to differentiate the one from the other. It seems chiefly owing to this property of the reagent that so much confusion exists as to the relation between protoplasm and callus in the sieve-plate of Gymnosperms; from what follows it will be seen that Russow, like later writers, was at first misled with regard to the callus.

In a transverse section of the sieve-tubes, he found that the mature radial walls are traversed by red-brown rods, swollen at the head (*geknöpften Stäben*) and interrupted at the middle lamella by nodules usually coloured yellow (Figs. 3, 4, and 5, Pl. XXXI, *gs. m.*). Each half of a rod is spoken of as a callus-cork (Fig. 5, Pl. XXXI).

The sides of the callus-corks (*Callus-Pfropfen*) have a dark edge or appear enclosed by dark brown striae, which he regards as being identical with the striae found traversing the callus-cushions of Angiosperms. These he calls the callus-

¹ Russow, Sitzber. der Dorpater Nat.-Ges., 1882, p. 264; trans., Ann. des Sc. Nat., Bot., 6^e sér., tome xiv, 1882, p. 173.

² Russow's callus reagent, a mixture of equal parts of chlor. zinc, iodine and a solution of iodine in potassium iodide.

rodlets (*Callus-Stifte*), which are in reality the slime-strings (Figs. 3 and 4, Pl. XXXI, *s.s.*).

The rods with swollen heads (the true callus-rods of the sieve-plate (Fig. 3, Pl. XXXI, *gs.*) are usually grouped together in twos and threes, and the portions of pit-closing membrane both between these groups and between the individual rods are coloured blue-violet by the callus reagent (Fig. 3, Pl. XXXI, *w.*).

The median nodules, which are seen to be equal to the rods in diameter, are not always sharply visible, but are usually stained yellow or brown. After the action of weak alcoholic potash the nodules may disappear or remain as irregular granules; the two halves of the callus-rods become separated owing to the swelling, and in some rare cases a thin thread was seen to unite the two separated callus-corks (Fig. 6, Pl. XXXI).

The nodules are still visible in sieves with a thick callus, but all traces of them disappear in sieves deprived of their callus. Russow asks, what do these nodules represent? but cannot give a satisfactory explanation; if they are portions of the middle lamella between the pit canals—the callus-corks,—then there is no communication, and yet a shutting of the sieves seems hardly likely. Janczewski also thought that they represented the middle lamella.

In the older callus-cushions Russow mentions the appearance of darker striae which diverge from the sieve-pores, which he regards as continuations of the substance of the rodlets of slime-strings (*Stiftsubstanz*) situated in the pores (Fig. 7, Pl. XXXI, *s.m.*).

He then mentions some interesting facts regarding the distribution of the callus-cushions, how that on the oblique terminal walls they are often one-sided and larger than elsewhere, whilst on the long walls each side of the plate may have a large cushion. With regard to sieves between medullary rays and sieve-tubes, he mentions their rarity, and that they are never perforated; callus-rods, however, are seen reaching to the lamella, but median nodules are said to be absent.

In a radial section of a sieve-plate, he sees the sieve-fields

covered by brown cushions, which, when examined in optical section, allow the callus-rods to be seen as dark patches on a clearer ground. The smaller the overlying callus-cushions, the more easily can the outlines of the callus-rods be seen through them (Fig. 2, Pl. XXXI).

He then discusses the contents of the tubes, and draws attention to the staining of the starch, which becomes a reddish-violet colour, whilst in the parenchyma it is a black purple; the pale staining is due to the presence of ferments, for starch stains in this manner in places where ferments are known to occur. This interesting observation will be referred to again later¹.

In his description of the development of the sieve-tubes he notices the primordial pits throughout the cambium, and that on the phloem side near the youngest mature sieve-tubes (the boundary-cells²) the surfaces of the pits are strewn with very fine points; he never, however, sees any 'threads' in a section of such a developing sieve-plate, but in the first thick-walled sieve-tube he notices yellow striae crossing the pit-closing membrane. Passing outward the callus appears in the second or third mature sieve-tube, filling the crenulations (or secondary pits) of the pit-closing membrane (Fig. 8, Pl. XXXI, *c.*), and stretching in towards the interior as very fine, often double brown stripes (Fig. 9, Pl. XXXI, *gs.*). In the next tube the stripes have become much thicker and now form the callus-rods whose extremities enlarge to form the callus-heads or cushions (Fig. 10, Pl. XXXI, *c.*). About or before this time the median nodules are first noticeable. Nothing is said about the origin of the rodlets—i. e. the slime-strings—which are enclosed in the callus-rod.

A further contribution to the subject of sieve-tubes is made by Russow³ in his well-known paper, 'On the perforation of the Cell-wall'; for by the use of the methods there described

¹ Cp. p. 594.

² The term 'boundary-cells' was used by Russow to designate the youngest layer of active sieve-tubes.

³ Russow, Sitzber. der Dorpater Nat.-Ges., 1883, p. 570.

the slime-threads which perforate the callus-rods can be seen extremely clearly, after solution of the callus, to be the connecting threads; and the granules also occurring at the middle lamella are found to be nothing more than thickenings or swellings of the connecting threads¹ (Figs. 4 and 5, Pl. XXXI). He also gives reasons why he considers the connecting threads of the sieve-tubes are not formed of protoplasm, because the protoplasmic threads appear finely granular in the parenchymatous cell-walls, whereas the connecting strings of the sieve-plate are throughout smooth and homogeneous.

A comparison of Russow's two sets of observations shows that what he formerly considered to be callus-rodlets (*Callus-Stifte*) must now be regarded as slime-threads or strings (*Schleimfäden*), since with the latter method the callus had been dissolved away by the sulphuric acid (Figs. 4 and 5, Pl. XXXII). He has also changed his views about the nature of the median granules, which he considered in the earlier paper to be portions of the undissolved pit-closing membrane, but now to be nothing more than swellings on the connecting threads. Thus, according to Russow, the sieve-plate is traversed by callus-rods and each rod encloses a group of slime-strings, which bear swellings or nodes at the middle lamella (Fig. 4, Pl. XXXII).

The various accounts of sieve-tubes given by Strasburger must now be considered. In the same year as Russow's first paper, he attempted to solve the problem of the development of the sieve-plate², and a short abstract of his results now follows:—'In the pit-closing membranes of primordial pits, glistening points, which are capable of swelling, are visible; the membrane between these spots then begins to thicken, thereby forming fine pores, which lead up to the swollen points and become filled with granular contents staining yellow in chlor. zinc. iodine solution; at the entrance to the pores this substance, which is highly refractive, forms a knob-like

¹ Russow compares the median nodes on sieve-tube-threads with what he considers similar swellings on the threads in bast-parenchyma-cell walls of *Fraxinus*.

² Strasburger, 'Bau und Wachstum,' p. 57 et seq., Figs. 33-43, Taf. iv.

swelling, and shortly afterwards the swollen places of the membrane are absorbed and the granular substance stretches across the membrane without a break.'

Strasburger finds these rods react as albumen, but thinks they may differ at different seasons of the year; Russow, however, who also describes this appearance of rods, considers them to be formed of callus. Strasburger then goes on to describe the formation of the callus-masses or cushions which, he states, arise as gelatinous-thickening layers of the thickened (that is the cellulose) portions of the pit-closing membrane, and are then added to by the protoplasm: fine radial lines are often seen in these cushions which appear to be fine canals.

A further contribution to the subject is made by Strasburger¹ in 1884 in his 'Botanisches Practicum,' where he describes the structure of the mature sieve-plates of *Pinus*, and gives figures which have been reproduced in nearly every recent textbook.

He used either Russow's callus reagent or water-blue, and his descriptions agree mainly with Russow's final results. He differs from Russow, however, since he considers the strings in the callus-rods to be formed of protoplasm, and the median nodules to be the swollen middle lamella of the sieve-plate. With regard to the formation of the callus-plates he seems to have reverted from his former position² to that of Russow³, for the head-like prominences of the sieve-fields are described as fusing together to form a plate⁴. The further development of the callus-plate is due to the protoplasm, and striae formed by the plasma-strings are often seen traversing the callus-cushions.

In the disused sieve-tubes all the callus is dissolved away, but the primary wall of the tube is left separating one sieve-

¹ Strasburger, 'Bot. Pract.', 1884 (German ed.), pp. 147-151, Figs. 63 and 64. Similar figures without the explanatory text are given in the third edition of the Practicum, 1897, and in Strasburger's recent textbook.

² 'Bau und Wachstum,' p. 59.

³ Sitzber. der Dorpater Nat.-Ges., 1882, p. 281.

⁴ 'Botanisches Practicum,' p. 151.

tube from the next and the median nodules are also still visible. Thus there is no open communication between adjoining sieve-tubes.

It is unfortunately by no means easy to elucidate Strasburger's meaning by the help of his figures, for the surface-views do not agree with the sections, because apparently the callus-rods, which should correspond to the sieve-fields, are not indicated in the figures of the tangential sections¹. It is also a difficult matter to see clearly all he describes when using his magnification. These results of Strasburger's, however, which agree so closely with those of Russow, will be shown later to correspond very nearly to the results obtained during the present research, far more closely in fact than any of the subsequent views which he has published.

The next paper bearing on the subject is that of Kienitz-Gerloff² to which only a passing reference need be made; he gives two very indifferent figures of what must be 'slime-strings' showing median nodes, for all the callus is dissolved away by the strong sulphuric acid he employed in his method.

Some new views on the structure of the sieve-plate were brought forward by Strasburger in his 'Leitungsbahnen'³, published in 1891, which differ a good deal from those already noticed.

In this work he deals more especially with the relations existing between sieve-tubes and albuminous cells, and this part has already been discussed in a former paper⁴.

Referring to the sieve-plates⁵ he agrees with Russow⁶ as to their formation, and states that the first change on the surface of the membrane is a fine puncturing, which appears as fine points arranged in nearly circular groups, and the separate groups form a field of the sieve-plate.

¹ 'Bot. Practicum'; cf. Fig. 63 with Fig. 64 A.

² Kienitz-Gerloff, Bot. Zeit., 1891, p. 34, and Fig. 31 A and B, Taf. i.

³ Strasburger, 'Leitungsbahnen,' p. 60 et seq.

⁴ Gardiner and Hill, 'Histology of Cell-wall,' pt. I. 'Connecting Threads in *Pinus*,' by A. W. Hill.

⁵ Loc. cit., p. 69 et seq.

⁶ Russow, loc. cit., 1882, p. 279; also cf. back, p. 579.

A comparison of surface-views and sections shows that the fine points correspond with plasma-filled pores; these threads thicken later on and increase in length as the wall thickens, and appear like his figure in the 'Practicum'¹. The threads do not stain well with aniline blue in this early stage, but this condition soon passes and the fine plasma-threads are changed into callus-substance; with this change the threads become thicker and form now the callus rods².

Referring to the median granules³ or nodules (*Knötchen*) situated at the middle of active sieve-plates, he inclines as before to the view that they are swollen places of the membrane, so that then their presence must indicate a closing of the active sieve-pores.

In the empty tubes, however, he, like Russow, is unable to recognize the median nodules, which is contrary to his former expressions of opinion⁴.

With the change of the threads into callus the highest point of sieve-tube function appears to be reached; the heads of the rods soon swell and fuse to form a callus-plate.

In the 'one-sided pits' between the albuminous cells and sieve-tubes he notices that on the sieve-tube side the callus-rods reach to the middle lamella, and that on the side towards the albuminous cell no callus exists, but very fine threads lead from the median granules to the albuminous cells⁵.

Within the last few days a copy of Strasburger's latest paper on 'Protoplasmic Connexions'⁶ has reached me, through the kindness of the author, in which he again deals with the sieve-plates of *Pinus*.

Alcohol material stained with water-blue is used and the results agree on the whole with those given in the 'Leitungs-

¹ 'Bot. Practicum,' 2te Aufl., p. 146, Fig. 64 A.

² Figs. 33 and 34, Taf. ii, 'Leitungsbahnen,' should be compared with the figures in his 'Practicum.'

³ 'Leitungsbahnen,' p. 71.

⁴ 'Bot. Practicum.' See back also, p. 582.

⁵ 'Leitungsbahnen,' Figs. 6-9, Taf. i; Fig. 35, Taf. ii. Jahrb. f. wiss. Bot., Bd. xxxvi, Heft 3, Taf. xiv, Fig. 28.

⁶ Strasburger, 'Ueber Plasmaverbindungen pflanzlicher Zellen.' Jahrb. f. wiss. Bot., Bd. xxxvi, Heft 3, pp. 523-526, Figs. 21-28.

bahnen.' Protoplasmic threads become changed into callus-rods, but the refractive nodules are not situated on these rods as formerly supposed, for they are now found to alternate with the callus-rods and to represent the meshwork of the middle lamella between the callus, and this meshwork remains after the solution of the callus. These conclusions were arrived at by the help of thinner sections and greater magnification than had been formerly employed. His experiments have been repeated—as have those of Russow, &c.—but, as will be seen later, my results do not at all agree with these latest views put forward by Strasburger.

In the paper on 'The connecting threads in *Pinus sylvestris*'¹ some figures of sieve-tube 'threads' are given: these, except in Fig. 29, are in reality the slime-strings with median nodes; the grouping of the slime-strings also is not well shown, and no callus is indicated. These discrepancies are due to the employment of the acid-violet method, which is not the best method for a research into the structure of the sieve-plate.

An important memoir was published by Perrot² in 1899 on sieve-tubes in general, which on the whole is a very useful *résumé* of previous research. His account of the structure of the sieves of Gymnosperms is, however, very imperfect and Russow's valuable work receives but scant attention at his hands.

To summarize then the most important views which are held concerning the structure of the sieve-plate:—Two distinct sets of opinions have been published, firstly, those of Russow with which Strasburger's 1884 account mainly agrees; and secondly, those given by Strasburger in his later works³.

Russow found that in the mature condition the sieve-plate is traversed by groups of slime-strings enclosed by callus-rods, and that at the middle lamella each slime-string bears a nodule or swelling, which disappears on the solution of the callus.

The callus, which is formed by deposition from the proto-

¹ Hill, loc. cit., Figs. 26-29.

² Perrot, 'Le tissu criblé,' 1899, pp. 43-47 and 67-72, &c.

³ 'Leitungsbahnen,' and Jahrb. f. wiss. Bot., 1901.

plasm, arises in the secondary pits on each side of the pit-closing membrane and grows towards the middle lamella, after which the heads of the rods enlarge to form the callus-masses. Russow thus differs from Wilhelm and Janczewski in his view of the formation of the callus, since they considered it was due to a change of the cellulose. According to Strasburger (1884) the fine strings enclosed in the callus-rods are 'plasma-filled canals,' and the median nodules correspond to swollen places of the middle lamella which remain after the solution of the callus; otherwise his account, which does not include developmental questions, corresponds with that of Russow.

The views recently put forward by Strasburger differ considerably from the foregoing. Protoplasmic threads are seen to cross the young pit-closing membrane, and then at a later stage they are transformed bodily into callus-rods; and the highly refractive, median nodules are found to be portions of the middle-lamella membrane between the rods of callus which persist in the old and empty sieve-plates. The callus-cushions are formed by the fusion of the heads of the rods, and are added to by the activity of the protoplasm.

Thus, according to Strasburger¹, it is the callus which is all-important for intercommunication between adjoining sieve-tubes, whereas Russow considers the slime-strings to be the necessary elements of the sieve-plate.

The existence of these different views must, I think, be attributed to the employment of Russow's callus reagent and water-blue, which stain both callus and protoplasm; and also to the use of alcohol material. It is impossible to study the development of the sieve-plate with these reagents, and it is only after the callus has been formed that any definite results can be obtained by their aid.

THE PRESENT RESEARCH.

The present research was accordingly undertaken, firstly, with the view of finding out which of the views already mentioned agreed with the observed facts; and secondly,

¹ Strasburger, 1891 and 1901.

whether by the use of Gardiner's methods any fresh light could be thrown on the development and structure of the sieve-plate.

Most of the work has been carried out in Mr. Gardiner's laboratory, and I must take this opportunity of acknowledging the help he has always given me by his invaluable advice and kind encouragement.

The material used in this research was obtained principally from the main trunk¹ of a tree of *Pinus excelsa*, about thirty years old, during June, July, and August, and was preserved in very small pieces by methods based on those already published by Gardiner². Staining was effected by the safranin method chiefly, and also by means of the acid-violet mixture, and a good contrast staining was produced by placing the safranin-stained sections in water blue, the slime-strings being stained red and the callus blue. The results obtained from an examination of the phloem of *Pinus excelsa* have been compared and found to agree with those from other species of *Pinus*, so that the following account may be taken as applying to the genus rather than to any species of *Pinus* in particular.

Transverse and radial sections of the phloem have chiefly been studied, which are of more value than sections cut tangentially, since the sieve-tubes are seen in chronological order, and the various stages in their history from their formation by the cambium to their ultimate disuse can be easily traced.

Before the more minute structure of the sieve-plate is considered, a few facts concerning the relation of the sieve-tubes to the cambium and the constitution of the cell-walls must first be noticed. The cambium is not a very clearly defined layer of tissue, since it passes—in the summer condition—insensibly into the xylem on the one side and into the phloem on the other. Between the developing and adult sieve-tubes, however, there is a sharp distinction, for the mature sieve-tubes possess peculiarly thickened glistening

¹ Russow found that such material gave the best results owing to the large size of the cells, and the numerous developmental stages present. Sitzber. d. Dorpater Nat.-Ges., 1881, p. 110; 1882, p. 259.

² Gardiner, Proc. Camb. Phil. Soc., 1898, p. 508.

cell-walls and the youngest layer of these active tubes, which was designated as the 'boundary-cell layer' by Russow, appears as a well-marked line limiting the zone of the active sieve-tubes (Fig. 1, Pl. XXXII).

These thick walls of the active sieve-tubes, which have been examined by Leger¹—whose experiments I have repeated—are found to be complex in structure. If the sieve-tube walls are acted upon with acid or basic dyes, such as Congo red or methylene blue, which stain cellulose and pectic substances respectively, it is found that the cell-walls of the young developing sieve-tubes are composed of pecto-cellulose like those of ordinary parenchymatous cells; soon, however, they become covered internally by a layer of pure cellulose, so that in a mature sieve-tube, as seen in a transverse section, there is a middle lamella of pecto-cellulose with a broad band of pure cellulose on each side. The pit-closing membranes of the active sieve-tubes show a similar structure (Figs. 2 and 3, Pl. XXXII). The structure of the mature sieve-plate, as will be pointed out later on, has an important relation to the complex nature of the cell-wall. A better idea of the changes which take place in the developing sieve-plates will, perhaps, be obtained if the structure of the mature sieve-plate is first described in detail. The pit-closing membranes or sieve-plates, which are somewhat lenticular in cross-section, are seen to have a circular or oval outline in surface-view with small angular or rounded areas, the sieve-fields scattered over the surface; in a section of the sieve-plate these fields are found to correspond to the callus-rods (Figs. 21 and 24, Pl. XXXIII).

Enclosed in the callus-rods are the slime-strings, some five to nine in number, as Russow describes; and at the middle lamella there may be seen either a single highly refractive median-nodule, dividing the callus-rod into two parts, or each slime-string may be seen to possess a median node—a result depending on the treatment the section has undergone. It thus appears that at the middle lamella there is a large refractive nodule, which is a part of the middle lamella,

¹ Léger, *Mém. de la Soc. Linn. de Normandie*, 1898, tome xix.

enclosing the median nodes which belong to the slime-strings (Fig. 11, Pl. XXXI; Fig. 20, Pl. XXXIII).

The Development of the Sieve-Plate.

The development of the sieve-plate can now be considered. It is found to be a matter of great difficulty to observe 'connecting threads' in the walls of meristematic tissues, and the radial walls of the cambium-cells unfortunately do not form an exception to this fact¹; and it is not until one examines the pit-closing membranes of developing sieve-tubes, about three cells away from the well-marked boundary-cells of the active phloem, that 'threads' can be observed with certainty.

The pit-closing membranes in this region are very thin, and the 'connecting threads,' which are arranged in small groups, are very fine, so that unless great care is taken in the staining of the sections, it is almost impossible to see them as fine lines at all. Each of these threads bears a small median dot like those previously described on the threads of cortical cells², and small granules are also frequently seen at each end of the threads, covering the surfaces of the young pit-closing membrane and so hiding the true ends of the threads (Fig. 8, Pl. XXXII).

As the pit-closing membranes are very delicate, it frequently happens that a slight swelling causes a separation of the wall to take place at the middle lamella, in which case the small median node is seen either on one side of the breach or else halved on both sides of the split middle lamella. Usually not more than two sieve-plates are seen in this youngest stage, for in the next sieve-tube, nearer the phloem, the pit-closing membrane is found to be rather broader and to have assumed a biconvex or lenticular shape, and to show a secondary pitting over its surface so that in sections its edge or margin appears slightly crenulated.

The 'connecting threads,' which are arranged in groups as

¹ Threads have, however, been clearly seen in the tangential walls of the medullary-ray cells right through the cambium.

² Hill, loc. cit., p. 108, Fig. 19, Pl. XXXIII.

already noticed, are apparently composed of protoplasm like those of the younger sieves, judging from their staining properties with iodine, safranin, and other dyes, and from their general appearance of granularity, &c.: they are as before quite fine and faint, and each thread possesses a small median dot (Fig. 8, Pl. XXXII (*b*)).

In the youngest thick-walled sieve-tubes¹, however—i.e. the boundary-cells (Fig. 9, Pl. XXXII (*a*))—‘the connecting threads’ may be similar to those of the younger elements just described, or, what is more likely, may show the commencement of certain secondary changes, which alter their appearance and staining properties in a striking manner (Fig. 9, Pl. XXXII (*b*)). In pit-closing membranes about this region the individual threads do not exhibit the uniformity of structure throughout their length which usually characterizes ‘connecting threads,’ for part of the thread is now much more noticeable, being thicker and staining more darkly than the unaltered portion of the protoplasmic thread. This change, which commences at the free surfaces of the threads, usually affects the halves of the threads on one side of the middle lamella first, and then in the same way attacks the halves on the other side; so that the young sieve-plates at this stage often present a peculiar appearance, with the portions of the threads in one-half of the pit-closing membrane fairly thick and darkly stained, whilst in the other half they are only faintly stained and much less conspicuous.

All stages in the alteration of the character of the sieve-plate threads have been noticed. The change is seen to commence on the surface of one side of the pit-closing membrane, and gradually to proceed inwards along each thread to the middle lamella; then the threads on the other side of the membrane appear to be similarly affected; and the change which can be easily observed in the stained sections

¹ Cf. pp. 579 and 587.

² A large number of sieve-plates at this border zone have been examined in sections of material, which has been fixed and stained in various ways, and in all cases similar results have been obtained, and the unsymmetrically stained threads have been clearly seen.

is seen to travel as before from the surface to the lamella. As a result of this the portions of the threads on either side of the lamella are again similar to each other, but they now stain more deeply, and present a more glistening appearance than the protoplasmic threads in the embryonic sieve-plate, so that they appear rather to be of the nature of strings of mucus or slime (Fig. 11, Pl. XXXII).

The Formation of the Callus-Rods, &c.

If sieve-plates in this boundary region are examined in sections which have been stained with water blue after the previous safranin staining, a change is also seen to be taking place in the nature of the pit-closing membrane immediately in the neighbourhood of the thread-groups, for the ingrowths of the darkly staining portions of the threads are seen to be situated in a small patch or area of callus, which is stained a sky-blue colour by this reagent (Fig. 17, Pl. XXXIII). This change of portions of the cellulose membrane into callus, which proceeds simultaneously with the alteration of the threads just mentioned, appears therefore to be due to the same cause as that which modifies their composition, and when these changes have reached to the middle lamella the final result is that the cylindrical rods of callus enclosing the groups of slime-strings are formed which mark the active condition of the sieve-plate (Figs. 18 and 20, Pl. XXXIII; cf. Fig. 11, Pl. XXXI).

Accompanying the change of the protoplasmic threads into slime-strings, and the formation of the callus-rods from the cellulose layers of the pit-closing membrane, a change occurs at the pectic middle lamella, which is marked by the appearance of the median nodule. The structure of this nodule, which has always been an object of much difficulty, was by no means easy to make out, for the callus-rod appears to be divided by it into two halves, and by ordinary methods it cannot be seen that it is traversed by the slime-strings. If, however, the region of the middle lamella is examined after various treatments, so that in some cases the highly refractive

nodule is disorganized, it can be seen that the slime-strings are not interrupted, but are continuous across the middle lamella, where they each possess a median node or swelling¹, (Figs. 4 and 5, Pl. XXXII) which is the somewhat enlarged node of the original protoplasmic thread². At this stage the sieve-plates are completely developed and show the mature structure already described, which is characteristic of all the sieve-tubes in the active region of the phloem.

The active sieve-tube region forms a broad zone of tissue some twelve to fourteen cells across, bounded internally by the 'boundary-cells' and externally by older sieve-tubes where the development of the callus-cushions is taking place (Fig. 1, Pl. XXXII). The relation between the slime-strings and the callus is not always easy to follow in the older sieves owing to the ease with which the callus swells with some reagents; for the orange G, for instance, which is used to wash out the safranin often causes the callus to swell so strongly that an erroneous impression of the structure of the sieve-plate is liable to be given, unless a careful comparison of the double-stained sections is made with other sections stained by water blue alone.

Both Russow³ (cf. Fig. 7, Pl. XXXI) and Strasburger mention the appearance of striae or threads in the large callus-cushions, and they are figured by Strasburger⁴, but I have found them very difficult to observe by their methods.

As the callus-cushion increases in size owing to the fusion of the heads of the callus-rods, the slime-strings contained in the rods are lengthened to keep pace with the callus-growth, so that an appearance of dark-red radiating lines traversing a blue callus-cushion is obtained on staining with safranin and water blue. With further increase in size of the callus the threads become broken up into granules and then can no longer be of any value for intercommunication (Fig. 14,

¹ It is clear from working through Russow's methods, and from his accounts, that he saw both the median nodule and the nodes of the slime-strings, but he apparently did not attempt to harmonize the two effects.

² Cf. p. 588.

³ Russow, loc. cit., 1882, p. 281.

⁴ Strasburger, 'Bot. Pract.', 1897, Fig. 103 B.

Pl. XXXII), and very soon after this the callus becomes more diffuse, stains faintly and finally is dissolved away.

The median nodules also lose their refringence in these old sieve-plates and are not so conspicuous when stained with iodine solution, and disorganization gradually takes place which on the solution of the callus becomes complete. Thus finally only the original network of the unaltered pit-closing membrane, which now stains deeply with methylene-blue, is left, and no trace of the original middle-lamella membrane, stretching across the meshes of the network or of the nodules, could be found (Fig. 13, Pl. XXXI). The old and disused sieve-plate appears therefore to be a distinct sieve with open pores, which is contrary to Strasburger's figure¹ where he shows the middle lamella of the pit-closing membrane stretching between the pieces of the unaltered radial wall, and so shutting off the sieve-tubes from each other.

The cell-walls of the older disused sieve-tubes are much thinner than those of the active zone, apparently through loss of water, and, like their pit-closing membranes, they stain quite deeply with methylene blue owing to the infiltration of pectic substances.

The Sieve-Plates in Surface-View.

The appearance of sieve-plates in radial sections has been described by De Bary², Strasburger³, Russow (Figs. 1 and 2, Pl. XXXI) and others, and figured by the two former investigators. It seems, from what I have been able to observe, that these descriptions and figures refer to optical sections of sieve-plates and not to surface-views between which there is some difference.

It has already been pointed out how difficult it is to see traces of the actual slime-strings in the callus-rods in transverse section, when Russow's or Strasburger's methods are employed, owing to the similar colouration their reagents give with protoplasm and callus; yet in the pictures of surface-

¹ Strasburger, 'Bot. Pract.,' 1897, Fig. 103 c.

² De Bary, 'Comp. Anat.,' Fig. 77.

³ Strasburger, 'Bot. Pract.,' 1897, Fig. 101.

views, given by them, some four or five darkly stained and fairly large points are shown in each sieve-field, which I have been unable to see when examining real surface-views by their methods. If, however, an optical section is taken, the dark points can be seen and appear to be nothing else but the enlarged median nodes of the slime-strings. By our methods this view receives confirmation, for in a real surface-view a certain number of fine, regular, faintly stained points can be seen arranged usually round the edge of each sieve-field (Fig. 15 A, Pl. XXXII); but on focussing, so as to obtain an optical section, the appearance of the sieve-plate changes, and the little fields, which usually appear more numerous near the lamella, are now seen to be occupied by fewer, larger, and more darkly stained dots than those of the surface-view. Thus, whereas at the surface some 4-9 slime-strings may be seen in each field, at the lamella only from 3-5 nodes occur in the smaller and more numerous areas (Fig. 15 A', Pl. XXXII).

The difference between the number of the sieve-fields in a surface-view and an optical section of the same sieve-plate explains the description given by Russow of the developing callus-rods, which often, he says, stretch towards the middle lamella as 'double brown lines'¹ (Fig. 9, Pl. XXXI). For a callus-rod, which appears as a single sieve-field at the surface of the sieve-plate, frequently divides, and at the lamella two sieve-fields, or callus-rods, as seen in section, are found. Thus if there are ten sieve-fields at the surface of a sieve-plate, it is quite likely that as many as fifteen may be seen when an optical section is taken.

This fact of the division of the callus-rods also explains why the slime-strings of the mature sieve-plate are frequently found running in obliquely placed groups in the pit-closing membrane (cf. Fig. 11, Pl. XXXII), since the callus-formation proceeds along the groups of connecting threads.

An appearance like that of Fig. 16, Pl. XXXII is often seen in an optical section of a mature sieve-plate stained with safranin alone: in this case the appearance of sieve-fields is

¹ Russow, loc. cit., 1882, p. 281, and cf. p. 579.

given by the darkly stained, irregular granules, and the dark dots seen in them are, as we have already seen, the real nodes of the threads.

An examination of a callus-cushion of an older sieve-tube in surface-view shows a large number of stained points scattered irregularly over the surface (cf. Fig. 14, Pl. XXXII), which are the ends of the slime-strings radiating out through the callus; an optical section shows appearances similar to, but less distinct than those already described.

The Cause of Sieve-Plate Development.

The structure of the sieve-plate has been described at some length in the preceding pages, and it now remains to be seen whether any explanation can be offered of the complicated developmental changes through which the sieve-plate passes before it attains to its mature condition. For we have seen that as soon as the alteration of the threads commences, the callus also appears in the form of little basin-shaped masses on the free surfaces of the small secondary pits, enveloping the altered portion of the 'connecting threads' (Fig. 7, Pl. XXXII; Figs. 17-20, Pl. XXXIII), and the further changes in both proceed simultaneously; moreover, the appearance of the median nodule is coincident with the completion of these changes; so that it seems only reasonable to suppose that these simultaneous changes are all connected together and must be due to the same or similar causes; and there seems to me no doubt but that they must be attributed to ferment-action.

The presence of ferments in sieve-tubes has long been known owing to their peculiar effect on the starch-grains in the tubes, which stain a pinkish colour in consequence when treated with iodine solution. Another excellent example of ferment-action in sieve-tubes is afforded by the large holes occupied by thick slime-strings in the sieve-plates of Angiosperms, such as *Cucurbita*, which have been bored out through the pit-closing membrane apparently along the lines of 'connecting threads'; and in addition to this evidence the final solu-

tion of the callus-cushions is a further indication that ferments play an important part in the life-history of the sieve-tubes.

A ferment then we must assume, which is generated by the protoplasm of a developing sieve-tube of *Pinus*, enters the little pits and proceeds to attack and bore out the 'connecting threads' of each group; as it works along the threads towards the lamella its corroding action can easily be traced by the alteration of their staining properties, and the increase in their thickness which it causes (Fig. 9, Pl. XXXII; Fig. 17, Pl. XXXIII); at the same time or shortly afterwards the ferment may be generated in the adjoining sieve-tube of the same age, and enlarge the pores of the sieve-plate in the same manner until the lamella is reached, and so give rise to the complete slime-strings (Fig. 11, Pl. XXXII). But whilst the ferment enlarges the pores on the sieve-plate, it also affects the cellulose of the pit-closing membrane around each thread, and the threads being close together a callus-rod results, enclosing each group of slime-strings (Fig. 20, Pl. XXXIII; Fig. 11, Pl. XXXI). At the middle lamella, however, the membrane is not composed of pure cellulose but of pecto-cellulose, and the result of the ferment-action in this region is the formation of the highly refractive nodule¹, which is thus seen to be strictly comparable in its mode of origin to the callus-rod formed from cellulose (Figs. 4, 10, and 11, Pl. XXXI).

¹ In this connexion some of the effects produced on the median nodules with reagents are interesting; they are stained darkly with iodine solution or Russow's callus reagent (Fig. 6, Pl. XXXII), and also by eosin (faintly), safranin, and benzyle blue, but with the two latter the staining appears to be of the nature of a surface colouration, and the colour is often so dark that the several nodules of the sieve-plate frequently look like a continuous, darkly stained median-plate (Fig. 11, Pl. XXXII). With water-blue alone they are unstained though very prominent, but after the action of twenty-five per cent. chromic acid or ten per cent. potash they are stained purple in most cases. They also stain a bright blue with methylene blue after a few hours' previous treatment with picro-nitric acid. After the action for some time of either ten per cent. potash, five per cent. nitric acid, twenty-five per cent. chromic acid, picro-nitric acid, or boiling water, they are still visible; but after the action of one per cent. sulphuric acid made pale pink with a drop of weak permanganate of potash or of about eighty per cent. sulphuric acid (Russow's second method) they cannot be distinguished.

A somewhat analogous case of ferment-action along 'connecting threads' or their canals is afforded by an examination of the endosperm of the germinating seed of *Tamus communis*, which was described by Gardiner¹ in 1897, and the figure (Fig. 13, Pl. XXXII) given here was drawn from one of his preparations. In a section of the endosperm innumerable fine threads bearing small median dots are seen to cross the thick cell-walls, but in the immediate neighbourhood of the developing embryo the pores or canals of the threads are being bored out, and the cell-walls are being gradually dissolved by a cellulose-dissolving ferment, and all stages of the process can be seen. The ferment passes into the threads from the protoplasm and travels along them, causing them to stain more deeply and to appear thicker and more granular than those which are unattacked. The cell-wall around each thread is also slightly affected, but though no callus reaction is given, still the cellulose is hydrated and is stained by dyes which do not colour the horny cellulose of the unattacked cell-wall².

The ferment usually enters the wall on one side only at first and works towards the lamella, and often more than one thread may be engulfed in its corroding action; when, or in some cases before, the region of the lamella is reached from the one side ferments may enter from the other side and eat their way to the lamella in a similar manner, with the result that symmetrical corrosion effects are produced (cf. Fig. 13, Pl. XXXII). The further action of the ferment, which leads to the ultimate solution of the cell-wall, may proceed in various ways, which however do not concern us here; it is clear, however, from the foregoing account that the effects due to undoubted ferment action in the cell-walls of the endosperm are to a certain extent strikingly similar to those which are produced during the development of the sieve-plate.

¹ Gardiner, Proc. Roy. Soc., 1897, p. 106 with diagram figure, Fig. 3.

² In a surface-view of a similar wall of *Tamus* endosperm, appearances like the surface-views of *Pinus* sieve-plates are also obtained, for when stained with safranin four to seven dark points—the enlarged threads of a group—are seen to be situated in a pink-stained area—the hydrated cellulose,—while the rest of the wall is white and clear.

The Origin of the Callus.

But to return again to the main subject, the development and mode of growth of the callus¹ now requires to be considered more closely, since two distinct views as to its mode of formation are held:

1. That it is due to mucilaginous degeneration of the cell-wall; and
2. That the protoplasm alone is concerned in the formation of callus.

The first of these two divergent views is due to Wilhelm², who was supported by Janczewski³, and later by Oliver⁴, who in his paper on the sieve-tubes of *Laminaria* brought forward a strong case in proof of the cellulose origin of the callus.

On the other hand, the protoplasmic origin of the callus has the support of Russow⁵, with whom it originated, Strasburger⁶, and Fischer⁷, whilst Rendle⁸ adopts a middle course and believes that the callus (which he describes in the 'vesicular vessels' of the onion) may be derived both from the cell-wall and from the protoplasm; and lastly, Moore⁹ brings forward a different theory, for he considers the callus to be proteid in nature and therefore of protoplasmic origin.

The facts brought to light during the present research make it appear highly probable that the middle course between these two opposed theories, as is so often the case, represents the true state of affairs, namely, that the callus may be formed both by alteration of the cellulose cell-wall and by

¹ For the bibliography of the subject see Zimmermann, Bot. Mikrotechnik, where references to Mangin's papers are given.

² Wilhelm, Beitr. zur Kenntniss d. Siebröhren-Apparates d. dicot. Pflanzen, 1880.

³ Janczewski, loc. cit.

⁴ Oliver, Ann. Bot., vol. i.

⁵ Russow, loc. cit., 1882.

⁶ Strasburger, 'Bot. Pract.', 1884.

⁷ Fischer, Ber. d. Deutsch. Bot. Ges., 1885, &c.

⁸ Rendle, Ann. Bot., vol. iii. Rendle's results appear to be of great interest, and it is hoped shortly to examine the vessels of the onion by our methods, in order to see if there is any connexion between the callus-patches and groups of connecting threads.

⁹ Moore, Journ. Linn. Soc., vol. xxvii, 1891. Moore's results do not appear to have received any confirmation.

deposition from the protoplasm; but no doubt in some cases it may only arise in the one way, as for instance appears to be the case in *Laminaria*¹.

The callus-rods have been shown to be due to the action of ferments on the cell-wall, by means of which the cellulose is changed into callus, whilst the large callus-cushions found on old sieve-plates appear to have been deposited by the protoplasm, since, however large the cushion may be, there is no diminution in size of the pit-closing membrane (Fig. 22, Pl. XXXIII).

A certain amount of evidence is also afforded in support of the wall-degeneration theory by an anomalous case of callus-formation, which was noticed in a transverse section of the phloem of *Pinus* (Fig. 25, Pl. XXXIII).

In two adjoining sieve-tubes, next to a medullary ray, the cellulose layers of part of the tangential and radial walls (next the ray) had undergone a change into callus. The callus, which stained in the usual way with water-blue, merged insensibly into the cellulose of the rest of the wall, and had no relation to any connecting threads. The effect produced, which is precisely similar to some figures given by Oliver², appears to be due to the action of a ferment, which for some unknown reason has brought about the mucilaginous degeneration of the cellulose³.

With regard to the protoplasmic origin of the callus, there seems no *a priori* reason why the protoplasm should not form callus just as easily as it can form cellulose, and indeed Gardiner⁴ has shown in the case of the mucilage-hairs of *Blechnum* that mucilage is formed in a similar manner to

¹ Oliver, loc. cit. The callus of the 'trumpet hyphae' of *Laminaria* is formed by the alteration of the cell-wall.

² Ibid., Ann. Bot., vol. i, Pl. VIII, Figs. 7, 8, 9, &c.

³ Mangin, Compt. rendus, cxv, p. 260. Callus is described as occurring in the membrane of cystoliths and other places, which seems to indicate that in these cases also callus is formed from the cell-wall.

⁴ Gardiner and Ito, 'Mucilage-secreting Hairs in *Blechnum* and *Osmunda*'; Ann. Bot., vol. i, pp. 33 et seq. (Figs. 40 and 43). Also on p. 39 A formation of callus on the transverse walls of the mucilage-cells is also described, which appears to be of protoplasmic origin. Cf. Figs. 34, 36, 41, and 43.

cellulose by deposition from the protoplasm, and judging from its reactions callus only appears to be a special type of mucilage or hydrated cellulose.

The protoplasmic origin of the callus was suggested by Russow in order to account for the vast amount of this substance, which is found in the older sieve-tubes of various plants, without any apparent diminution in size of the original pit-closing membrane taking place; and although there is no doubt that a small callus-cushion can swell enormously without any addition to its substance¹, swelling in itself does not offer a sufficient explanation of the observed facts, and it seems likely that the protoplasm found in the older sieve-tubes adds to the callus-mass which has been formed in the first place from the cell-wall (Fig. 7, Pl. XXXI; Fig. 14, Pl. XXXII; Fig. 22, Pl. XXXIII).

An interesting case of the protoplasmic origin of the callus was also noticed in a section of the phloem of *Pinus excelsa*, where callus had been formed against the tangential wall of a medullary-ray cell; the wall appeared quite normal and was traversed by threads in the usual manner, but a convex pad of callus, equal in thickness to the wall, had been developed on the phloem-side of the membrane; as the original wall was unaltered the protoplasmic origin of the callus-pad seemed to be the only possible explanation² (Fig. 27, Pl. XXXIII).

In cells of this same medullary ray nearer the cambium-region, a peculiar appearance of the starch-grains was noticed, after staining the section with water-blue; for here and there in the cell-protoplasm fairly large crescentic bodies were seen, which stained a pale sky-blue, the colour being just like that assumed by the callus under the same conditions. These bodies appeared to be starch-grains undergoing mucilaginous degeneration (Fig. 26, Pl. XXXIII).

¹ By the action of orange G., the callus-cushions and the free ends of the callus-rods swell to nearly twice their former size.

² The section, which was a thin one, was shaken up several times in water with fine sand in order to see if the callus was not some foreign inclusion; but it could not be dislodged, and appeared definitely to belong to the wall in question.

I do not wish to lay too great stress on the two anomalous cases of callus-formation which have been brought forward, but I think they are not without value, as affording evidence of the way in which the callus may be formed in the one case from the cell-wall, and in the other from the cell-contents in connexion with the protoplasm.

The Albuminous Cells.

Before concluding this paper the peculiar connecting threads between the albuminous cells and the sieve-tubes, which were discussed at some length in a former paper¹, must be briefly noticed again here.

The youngest walls, in which threads could be seen, occurred within the limits of the boundary-cells of the phloem, and showed characters exactly similar to those of the pit-closing membranes of the very young sieve-tubes (Fig. 12, Pl. XXXII; cf. also Fig. 8); but about the region of the boundary layer of the sieve-tubes changes are seen to commence which result in the development of the unequally divided pit-closing membranes so characteristic of the albuminous cells², for on the sieve-tube side of the lamella the membrane thickens or swells to quite twice its original breadth, and the halves of the threads correspondingly increase in length, whilst on the side of the albuminous cell no great increase in size occurs; the middle lamella is thus much nearer to the albuminous cell-cavity than to the lumen of the sieve-tube, and the appearance of the unequally divided membrane is given. The shorter portions of the threads retain their protoplasmic character throughout their existence, and stain darkly like the ordinary medullary ray cell-threads; but the longer portions, on the sieve-tube side, pass through precisely similar phases of development to those which have been followed for the

¹ Hill, loc. cit., pp. 94, 118, &c., Figs. 8, 31, 36, 37. The figures of the sieve-areas of the albuminous cells in the stem are not very clear, owing to the callus having been omitted.

² Cf. figures in Strasburger's 'Leitungsbahnen,' 1891, and 'Plasmaverbindungen,' 1901.

threads of the sieve-plate; for ferments travel along the threads from the sieve-tube, converting them into slime-strings, and, at the same time, acting on the wall in their immediate vicinity form both the callus-rods and also the nodules at the middle lamella. The heads of the callus-rods swell with great ease or else are added to by deposition from the protoplasm, for it is a common thing to find quite large cushions of callus covering the sieves communicating with the albuminous cells, in places where no callus-cushions are developed over the sieve-plates (Fig. 23, Pl. XXXIII).

The difference between the character of the 'threads' on the two sides of the lamella is clearly shown by their staining reactions, for the short protoplasmic threads appear thick and stain deeply with safranin, whilst the longer slime-strings into which the sieve-tube portions of the threads have been converted appear thinner and stain only faintly with this dye (Fig. 12, Pl. XXXI; Fig. 12, Pl. XXXII). The appearances produced are exactly like those found in the albuminous cells of the leaf, where a precisely similar orientation of the thread-groups is found to occur¹.

A striking difference, however, occurs between the albuminous cell-sieves in the stem, and those in the leaves; for in the stem the slime-strings of the broader portion of the membrane traverse callus-rods, and are in every way similar to the threads of the sieve-plate; but in the leaf, although the longer threads appear to be of the nature of slime-strings, there is no callus-development associated with them².

¹ Hill, loc. cit., Figs. 8 and 36. Fig. 36 is not a good reproduction of the original figure.

Excellent examples of these unequally divided pit-closing membranes, showing the two kinds of thread, are found in *Dammara australis*; they appear to agree in all respects with those described for *Pinus*. Cf. Strasburger, 'Leitungsbahnen,' Fig. 35, &c.

² Numerous experiments were tried to see if the pit-closing membranes between albuminous cells and sieve-tubes in the leaf gave any indication of callus. Leaves of all ages were examined (one, two, and three years old), and the following stains were used: Congo red, the albuminous cell-walls stained, but the thick pit-closing membrane stained less deeply than the general wall; callus reagent, pit-closing membrane pale-violet; corallin soda and aniline blue, membrane unstained.

The portions of the peculiar lenticular patches in which these threads occur are, however, a good deal swollen, and appear to be but slightly removed from callus-substance, and it seems a reasonable view to consider that this swollen portion of the membrane is composed of a substance intermediate in composition between cellulose and callus¹.

The state of the albuminous cell-sieves thus makes it clear that it is only from the sieve-tubes that the influences, which so affect the character of the connecting elements, can emanate, and moreover, as was implied earlier in this paper², it seems evident that the sphere of influence of each sieve-tube only extends to the limits of its own cell-wall, that is to say, as far as the median nodes of the connecting threads.

Between the sieve-tubes and bast-parenchyma cells of *Pinus* there are no connecting threads³, so that the albuminous cell thread-groups are the only means of protoplasmic union between the sieve-tubes and the parenchymatous tissues of the phloem.

The Distribution of the Threads.

In the former paper⁴ the general distribution of the connecting threads was discussed especially with reference to their abundance in the radial walls of the various tissues. In the phloem this arrangement is even more conspicuous, for all the sieve-tube and albuminous-cell 'threads' occur in the radial walls, and only the medullary-ray cells possess them in their tangential walls; thus no direct communication by means of threads can take place between an older and younger sieve-tube in the same radial row.

According to the views recently put forward by Gardiner⁵ threads, or their rudiments, should be visible in all cell-walls, since the nodes of the achromatin fibres of the nuclear spindle

¹ Cf. Hill, loc. cit., Fig. 37; cf. also the hydrated cellulose of *Tamus*, v. p. 596.

² Cf. Fig. 9, Pl. XXXII, and Figs. 17-20, Pl. XXXIII, where the alteration of threads in the sieve-plates is seen to proceed from both sides of the membrane towards the middle lamella.

³ Hill, loc. cit., p. 110, and Fig. 24, Pl. XXXIV.

⁴ Loc. cit., p. 119.

⁵ Gardiner, Roy. Soc. Proc., 1900. Various examples are brought forward to show, in some cases, all the nodes prolonged as threads, or all overlaid, or else some nodes overlaid while the others are continued.

persist and form the nodes or median dots of the connecting threads.

When the formation of the sieve-tubes of *Pinus* is considered, it is seen that they arise by tangential division from the cambium, and therefore it is especially in the tangential walls that threads or their rudiments would be expected to occur.

In order to see if this was the case, numerous sections of tissues of all ages preserved in various ways have been examined, but with at present very little result; so that it is possible that the nodes may get obliterated very quickly, and only have an ephemeral existence in the developing cell-wall. It is quite likely, however, that they do occur in the walls, but that owing to imperfect methods of fixation or staining and the delicacy of the tissues, their presence has not yet been demonstrated. Their apparent absence does not in any way invalidate the theory which has been put forward, for the obliteration of threads owing to mucilaginous degeneration has been proved to be a phenomenon of common occurrence¹.

The Function of the Connecting Threads and Slime-Strings.

Theories as to the probable functions of connecting threads have frequently been put forward by previous investigators, which are in the main true, although in some cases the real threads on which their theories are based have never been seen. The value of the threads for the transmission of stimuli, and as paths for the passage of water and substances in solution, has been discussed and supported by special examples in the former paper, and there seems to be no doubt that the connecting threads are all-important for transmitting stimuli from cell to cell².

With reference to their function in translocation, it is difficult to bring forward direct proofs of their value, since their small size makes it almost impossible for any direct

¹ As, for instance, in the walls of the separating palisade-cells and other tissues. Cf. Hill, loc. cit., Figs. 7 and 11.

² Cf. Gardiner, Czapek, &c.

experiments to be performed, and Pfeffer¹ doubts whether the threads are of any use for the purpose. Brown and Escombe², however, have recently attacked the problem from the physical side, and have come to the conclusion that, given certain conditions, 'the flow of the diffusing substance may go on almost as rapidly through a "multi-perforate septum"—such as that provided by a pit-closing membrane studded with threads—as if no closing membrane were present.' Therefore—and these remarks apply with equal weight to the slime-strings of the sieve-plate—it seems highly probable from the study of the general distribution of connecting threads throughout the tissues and from a consideration of their enormous numbers, that their cumulative effect must be of great importance in translocation.

The slime-strings of the sieve-tubes of Angiosperms, owing chiefly to their size and position, have always been regarded as important factors in the translocation of the elaborated food materials, and there is no doubt but that the smaller ones in the sieve-plates of *Pinus* perform similar functions to those of other plants.

The present research, however, has yielded results which seem to prove that a passage of substances in solution can and does take place by means of the protoplasmic thread³. The mere fact that the thread of the developing sieve-plate becomes converted into the slime-string of the mature sieve,

¹ Pfeffer, 'Physiology of Plants' (Eng. ed.), 1899, p. 112, sect. 20. He gives an erroneous figure of 'connecting threads,' and there is no reference to any of Gardiner's work, an omission which should have been rectified by the editors of the English translation. The text is therefore largely based on the incorrect results of Kienitz-Gerloff's papers.

² Brown and Escombe, Phil. Trans. Roy. Soc. B., vol. cxiii, 1900, pp. 280, 281. Measurements of 'connecting threads' have been given in the paper on *Pinus*, Gardiner and Hill, pt. i, Phil. Trans. Roy. Soc. B., vol. cxiv, pp. 87, 88. The approximate dimensions of the individual threads appear to be in length 1.8 μ , and .3 μ in diameter. In the thin tangential wall of the medullary ray cells, in the cambium of *P. excelsa*, each thread was not more than 1.6 μ long, and in such a wall they appeared to be so arranged that about nine threads occurred in every 4 sq. μ of area.

³ Cf. the action of the ferment on the cell-walls and threads of *Tamus* endosperm.

all stages of which can be followed, is sufficient evidence to show that something has travelled along it; but the discovery, that this change can apparently proceed from a sieve-tube only as far as the middle of each thread¹, seems rather to point to the absence of any real and continuous communication between developing sieve-tubes by means of the connecting threads of the young sieve-plates.

A reason then for the boring out of the connecting threads by ferments seems naturally to suggest itself, namely, that open canals or pores may be formed which shall afford a means of direct and rapid communication between adjoining sieve-tubes. Whether the theory that there is no continuous intercommunication along a connecting thread is, in reality, a fact, and whether it applies to all connecting threads is a matter of extreme difficulty to investigate owing to the minuteness of the problem involved; but even should there be a minute imperforate membrane separating the two halves of a connecting thread, it would be the thinnest portion of the pit-closing membrane across which diffusion could rapidly take place.

The Value of the Callus.

The callus has been thought by some writers² to be the most important and essential part of the sieve-plate, for the rods of callus were considered as the actual connecting elements; but it seems to have been established beyond a doubt from the present researches that the slime-strings, which were first noticed by Russow, afford the true and only means of direct intercommunication between adjoining sieve-tubes. It remains then to be seen if any explanation can be offered to account for the presence of the callus-rods, and whether any function can be assigned to these conspicuous and invariable associates of the slime-strings.

The origin of the callus-rod, which is due to the action of the ferment on the cellulose cell-wall and of the median

¹ See back, pp. 589, 595, 600-2, on the development of the sieves between sieve-tubes, and between sieve-tubes and the albuminous cells.

² Janczewski, loc. cit. Strasburger, 'Leitungsbahnen,' 1891, and 'Plasmaverbindungen'; Jahrb. f. wiss. Bot., 1901.

nodule, which appears to be its homologue at the pectic middle lamella, has already been discussed, but the question of their physiological importance is a more difficult matter; with regard to the latter two views seem to be possible, either that the callus-rod and median nodules are formed because they are of real value to the active sieve-tubes, or because on the contrary the ferment which is at work on the canals of the connecting threads cannot help affecting the cell-wall in their vicinity.

As far as one can see, the first of these two suggestions is the correct view, for the action of the ferment, which gives rise to the sharp and clearly-defined callus-rods and nodules, is found to be so distinctly localized and circumscribed. It therefore seems likely that the callus, which swells so easily, may be a kind of spongy lining to the canals of each slime-string, and may regulate when necessary the dimensions of the pores of the active sieve-plates.

The chief function of the callus-cushions, which are developed towards autumn, and through which the slime-strings are continued, is also apparently to bring about the diminution in size of the canals of the slime-strings, and thereby to gradually slow down and eventually stop the processes of translocation between adjoining sieve-tubes. With the disuse and death of the sieve-tubes the callus and nodules are dissolved away and openholes are left between the empty cavities of adjoining tubes.

SUMMARY.

In conclusion it will perhaps be useful to give a brief summary of the results which have been arrived at during this research.

In the earlier part of the paper a *résumé* and criticism of the work of former investigators is given, and it is shown that the results obtained by Russow harmonize in the main with those which are brought forward in this paper, for he found that the mature sieve-plate is traversed by callus-rods which enclose strings of slime.

Much of the confusion which has existed up till now about

the structure of the sieve-plate can be attributed to the unsatisfactory nature of the reagents employed, since they do not differentiate between protoplasmic and callus structures; and for this reason the development of the sieve-plate could not be understood.

The youngest sieve-plates which could be examined showed 'connecting threads' like those in parenchymatous tissues; but in the so-called 'boundary cells'—i.e. the youngest thick-walled sieve-tubes—a change takes place; for a ferment apparently travels along and bores out the threads, converting them into slime-strings, and at the same time so affects the surrounding cellulose that the formation of the callus-rods results; and at the middle lamella, owing to the same cause, the median nodule appears enclosing the nodes of the slime-strings.

The effect produced by the action of ferments on the threads in the endosperm-walls of germinating seeds of *Tamus* is described, and its similarity to the state of affairs found in the developing sieve-plates of *Pinus* is pointed out, for the two cases appear to be analogous.

The development of the sieves between the albuminous cells and the sieve-tubes has also been worked out, and it has been found that the portions of the threads on the sieve-tube side of the middle lamella undergo changes precisely similar to those described for the sieve-plates, whilst the shorter portions on the cell-side of the lamella retain their protoplasmic character.

The two rival theories as to the formation of the callus are also considered, and reasons are given for supposing that it may arise either by mucilaginous degeneration of the cell-wall or by deposition from the protoplasm.

The paper closes with a discussion as to the value of 'connecting threads' for translocation, about which it is difficult to come to a definite conclusion; the slime-strings, however, do seem without a doubt to be of great importance as translocatory channels between adjoining sieve-tubes.

In conclusion, it is suggested that the callus may be of value

in regulating the size of the pores of the active sieve-plate, and that the further development of the callus-cushions attenuates and finally closes them altogether. When this has occurred the activity of the sieve-tube is at an end, and it then becomes a dead and empty member of the phloem.

EXPLANATION OF FIGURES IN PLATES XXXI, XXXII, AND XXXIII.

Illustrating Mr. A. W. Hill's paper on the Sieve-Tubes of *Pinus*.

PLATE XXXI.

Diagrammatic Figs. 1-10, to illustrate Russow's views.

Fig. 1. A sieve-plate in surface-view, showing the sieve-fields (*f.*) with the sieve-pores or canals (*sp.*).

Fig. 2. A sieve-plate in optical section; showing the outlines of callus-cushions (*c.*) enclosing the darker cross-sections of the callus-rods (*f.*).

Fig. 3. A sieve-plate in section, showing the arrangement of the callus-rods in groups, the membrane (*w.*) between the rods is stained violet with chlor. zinc. iod. Some of the callus-rods (*g.s.*) show dark edges. ('Callus-stifte.') (*s.s.*)

Fig. 4. Diagrammatic view of the complex callus-rods, showing the sieve-pores (*p.*) and canals with the rodlets (*s.s.*) and the median granules (*m.*). In his 1883 paper Russow shows that the rodlets are slime-strings (cf. Fig. 5, Pl. XXXII).

Fig. 5. A callus-rod (*g.s.*) enlarged, showing the two callus-corks or -rods ('Callus-propfen').

Fig. 6. After treatment with potash the two corks or rods (*r.*) have separated, and appear united by a fine thread.

Fig. 7. A callus-cushion (*c.*), showing lines radiating from the rodlets, which consist of 'Stiftsubstanz' (*Ss.*), the radiating lines are the 'Stiftmassen' (*Sm.*).

Fig. 8. The development of the callus (*c.*) in the form of refringent particles, which arise in the crenulations of the sieve-plate; traces of the 'connecting threads' (*c.t.*) are also seen.

Fig. 9. The formation of the callus-rods (*g.s.*); in some cases the appearance of the 'double-brown stripes' is seen.

Fig. 10. The development of the callus-cushion (*c.*) owing to the swelling up of the heads of the rods.

Diagrammatic Figs. 11-13, illustrating the present research.

Fig. 11. A mature sieve-plate, showing callus-rods (*r.*) and nodules (*m.n.*) at the middle lamella (*m.l.*) enclosing the slime-strings (*s.s.*) with their median nodes (*m.*).

Fig. 12. A sieve between a sieve-tube and an albuminous cell. On the sieve-tube

side of the lamella (*m.l.*) are seen callus-rods and slime-strings, but on the albuminous cell-side (*alb.c.*) groups of protoplasmic connecting-threads (*c.t.*) occur.

Fig. 13. An empty sieve-plate, showing open pores.

PLATES XXXII AND XXXIII (*Original Figures*).

The lenses used were Swift's $\frac{1}{8}$ and $\frac{1}{12}$ apoc. with b. and c., and 6, 8, and 12 compensating oculars.

PLATE XXXII.

Pinus excelsa.

Fig. 1. A microphotograph of a transverse section through the phloem (*p.*), cambium (*c.*), and xylem (*x.*) of a piece of material of *Pinus excelsa* cut from the main trunk. The gradual passage from the cambium to the xylem is seen, and on the phloem side the sharp line caused by the boundary layer of sieve-tubes (*b.c.*) is clearly marked. The sieve-plates (*sp.*) can be seen as small dark patches on the radial walls of the sieve-tubes (*st.*).

The cells with dark contents are either bast-parenchyma cells or sieve-tubes filled with resin.

The medullary rays (*mr.*) are also clearly shown. The youngest sieve-plates in which threads have been seen occur just on the cambium side of the boundary layer.

Fig. 2. A small piece of the phloem-tissues in transverse section stained with Congo red. The cellulose portion of the wall is stained deep pink, whilst the pecto-cellulose lamella and medullary ray cell-wall, &c. only stain pale pink.

Fig. 3. A similar section to Fig. 2 stained with watery methylene blue; the younger cellulose layers are scarcely stained, the middle lamella is deep blue and the medullary-ray walls, which are less pectic than the lamella, a pale violet-blue. These two figures should be compared with the diagrammatic figure (Fig. 11, Pl. XXXI).

Fig. 4. A sieve-plate in transverse section, showing slime-strings with median nodes, and a slight indication of callus-rods—prepared from fresh material by Russow's method—the sulphuric acid (eighty-one per cent.) only used momentarily. ($\times 750$.)

Fig. 5. A section of a sieve-plate as above, after longer treatment with sulphuric acid. ($\times 1000$.)

Fig. 6. A fresh section examined in Russow's callus reagent; slime-strings and median nodules are seen, but the callus-rods were not well defined. ($\times 500$.)

Fig. 7. A young sieve-plate in transverse section stained with water-blue; the callus (*k.*) is seen growing towards the middle lamella in finger-like processes, and between these rods protoplasmic threads with median nodes are dimly seen. ($\times 1000$.)

Fig. 8. Transverse section across the sieve-tubes of the boundary region, showing three sieve-plates, the youngest (*a*) shows fine protoplasmic threads in groups, each thread with a median dot; the one in the middle (*b*) is somewhat thicker, also with protoplasmic threads; whilst the oldest (*c*) shows slime-strings with a darkly stained, median granule (stained by acid-violet method). ($\times 750$.)

Fig. 9. Transverse section of young sieve-tubes, showing sieve-plates in the transitional stage; the youngest (*a*) is as yet unacted on by ferments, but in both

(*b*) and (*b'*) ferment action is taking place by means of a ferment which has originated in the tube (*B*). The presence of the ferment is indicated by the darker staining of the half-threads. The sieve-plates show secondary pits, and in the older membranes (*c*) slime-strings and callus-rods have been formed (stained with safranin). ($\times 750$.)

Fig. 10. Tangential section of a young sieve-tube before the appearance of the callus, fine protoplasmic threads cross the sieve-plates. ($\times 750$.)

Fig. 11. A mature sieve-plate in transverse section, the crenulated pit-closing membrane with slime-strings is clearly seen, also the median nodules. The callus-rods are not indicated (stained with safranin). ($\times 750$.)

Fig. 12. Transverse section of the phloem in the region of the boundary cells, showing young albuminous cells of the medullary ray with pits between them and sieve-tubes.

The pit-closing membrane (*a*) is thin with fine protoplasmic threads, whilst in the next cell secondary changes have occurred; and the threads on the albuminous cell-side of the middle lamella are protoplasmic, whilst on the sieve-tube side they are slime-strings (stained with safranin). ($\times 1000$.) (Cf. Fig. 12, Pl. XXXI.)

Fig. 13. *Tamus communis*, endosperm. A section of the cells of the endosperm bordering on the developing embryo; ferment action is seen to be taking place and various stages are figured; in some places the ferment is just entering the wall, in others a good deal of disorganization at the lamella has occurred. The entry of the ferment from one side is well seen. (From a section prepared by W. Gardiner and stained with safranin in 1896.) ($\times 750$.)

Fig. 14. An old callus-cushion in transverse section, showing numerous threads which have become broken up into granules. ($\times 750$.)

Fig. 15. *A*. A young sieve-plate in surface-view, showing the ends of the slime-strings as fine dots in a few rather large sieve-fields.

A'. The same plate in optical section, showing smaller and more numerous fields with dark dots which are the median nodes of the slime-strings. ($\times 750$.)

Fig. 16. A common appearance of mature sieve-plates as seen in optical section. The appearance of sieve-fields is given by the darkly stained, irregular, median granules, and the nodes of the slime-strings are visible as dark dots; cf. with Fig. 11.

PLATE XXXIII.

In all the figures of this plate the callus is indicated by a blue tint to represent the colour obtained after staining with water-blue.

Fig. 17. The callus is seen arising on one side of the membrane, and the accompanying alteration in the character of the threads is also indicated. ($\times 750$.)

Fig. 18. A further stage in the process, for here the callus-rods have reached the middle lamella, and the median nodules have been formed. Cf. Pl. XXXI, Fig. 9.

Fig. 19. The callus arising on each side of the membrane.

Fig. 20. A mature sieve-plate, showing callus-rods, slime-strings, and median nodules. (Stain, safranin and water-blue.) ($\times 750$.)

Fig. 21. A mature sieve-plate, showing the callus-rods stained with water-blue alone. ($\times 1000$.)

Fig. 22. An old sieve-plate with a much-swollen callus-cushion; the callus-rods are seen in the pit-closing membrane. ($\times 750$.)

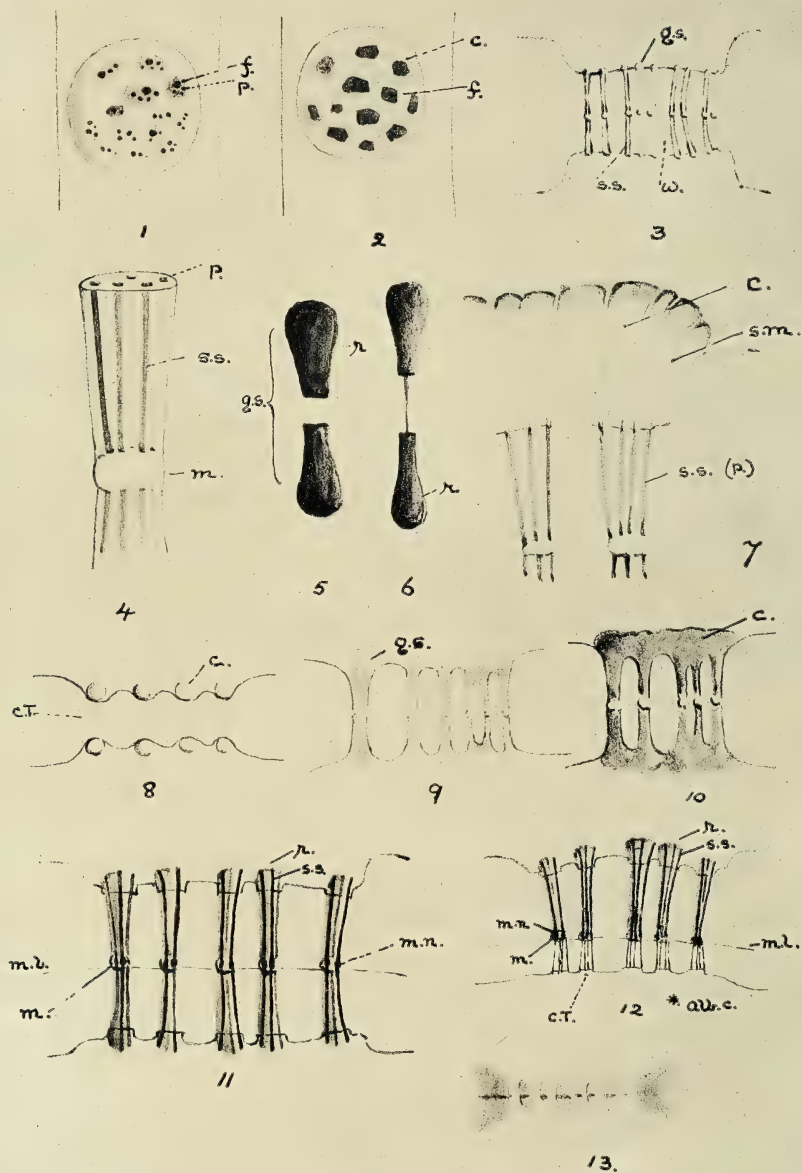
Fig. 23. A pit between a sieve-tube and an albuminous cell showing callus formed on the sieve-tube side only, and reaching to the lamella. Cf. Pl. XXXI, Fig. 12. ($\times 750$.)

Fig. 24. Sieve-plates in radial sections of the phloem, showing sieve-fields in surface-view.

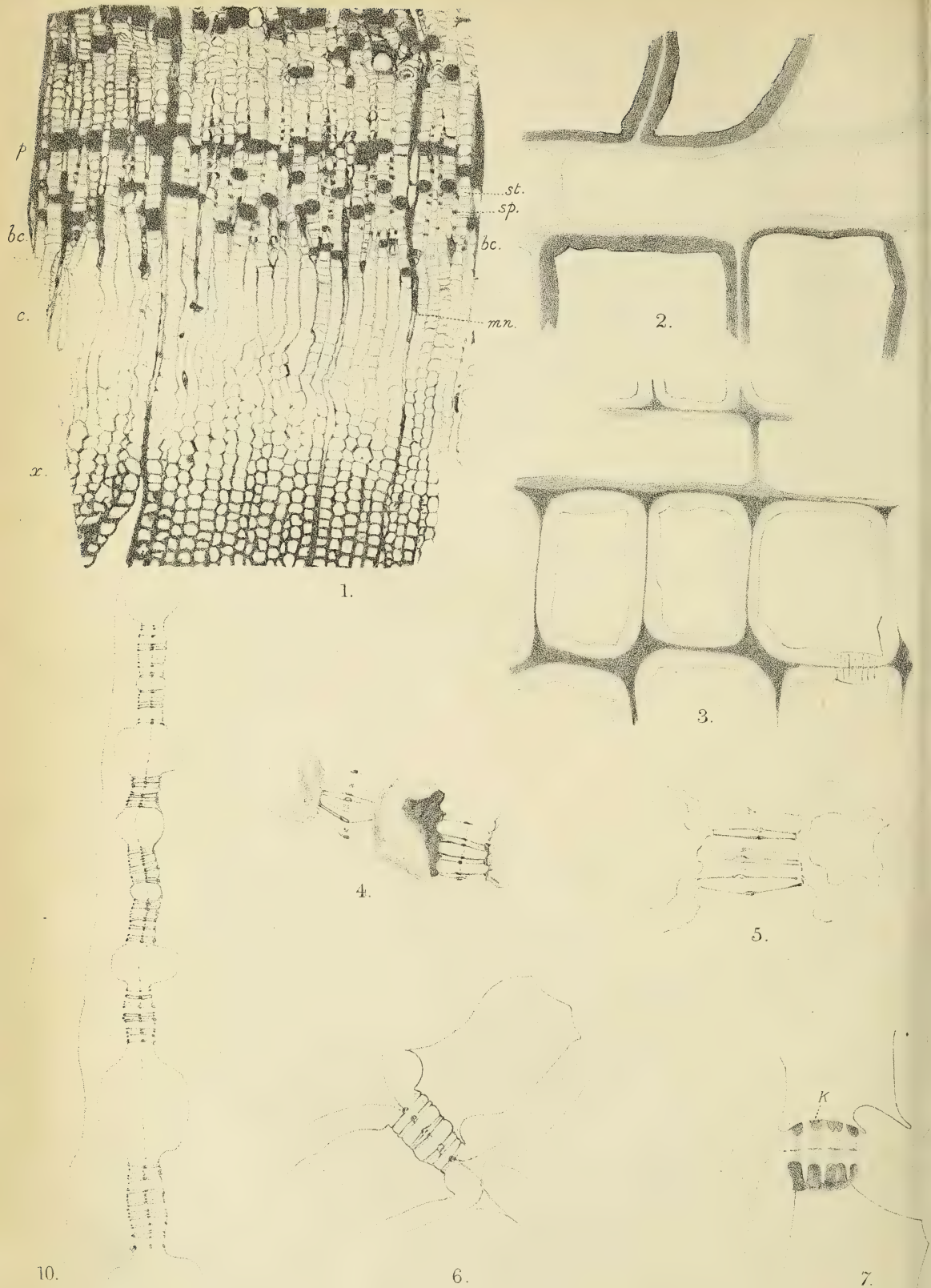
Fig. 25. Transverse section of the phloem, showing an anomalous formation of callus (*k*.) owing to the alteration of the cellulose layers of part of the tangential and radial walls of two contiguous sieve-tubes. At (*b*.) a mature sieve-plate is seen, and at (*mr*.) a medullary ray with threads in a tangential wall.

Fig. 26. A medullary ray cell stained with safranin and water-blue, showing starch-grains giving the callus reaction.

Fig. 27. An older cell of the same ray, showing a formation of callus (*k*.) against the tangential wall on the side towards the cortex. ($\times 750$.)



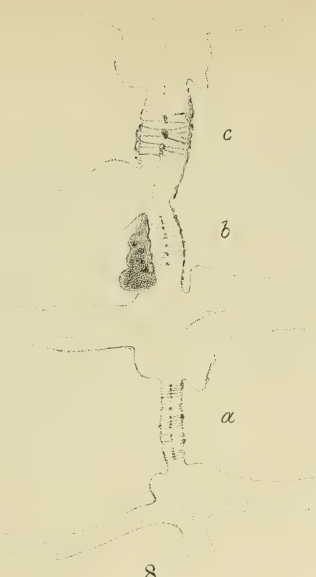
A. W. HILL.—SIEVE-TUBES OF PINUS



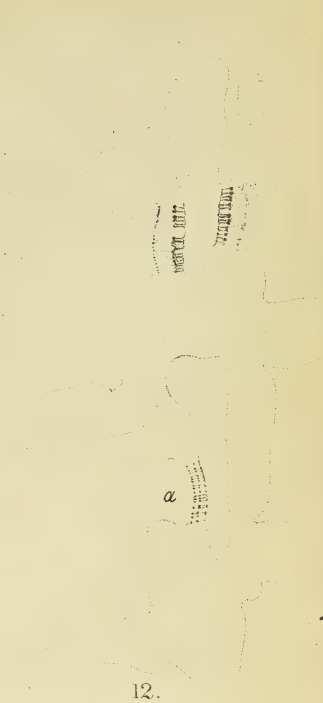
A. W. H. del.



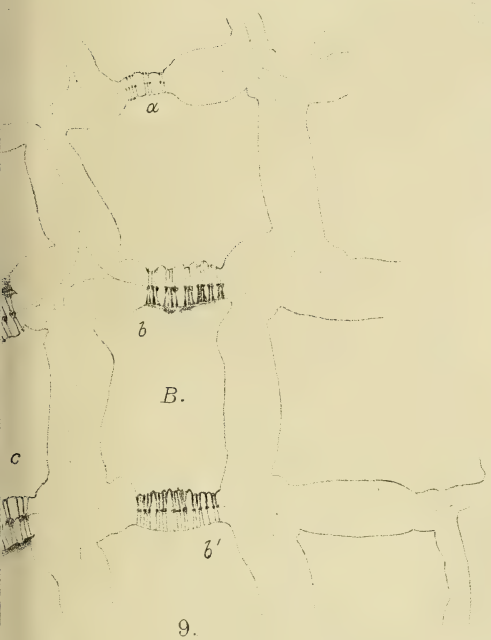
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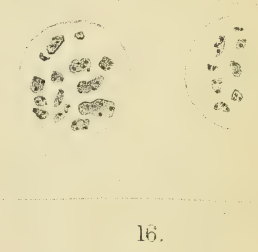
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15.



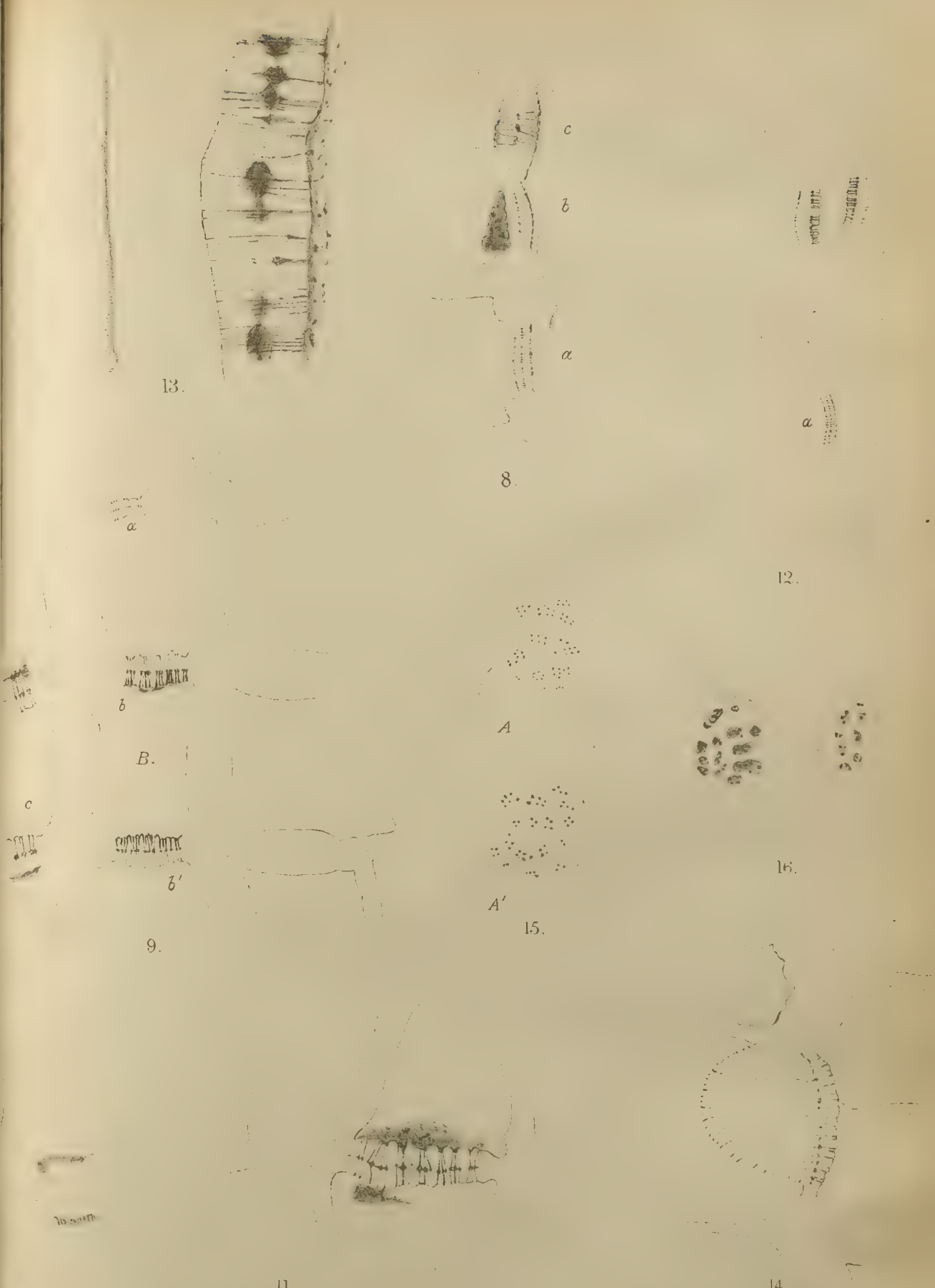
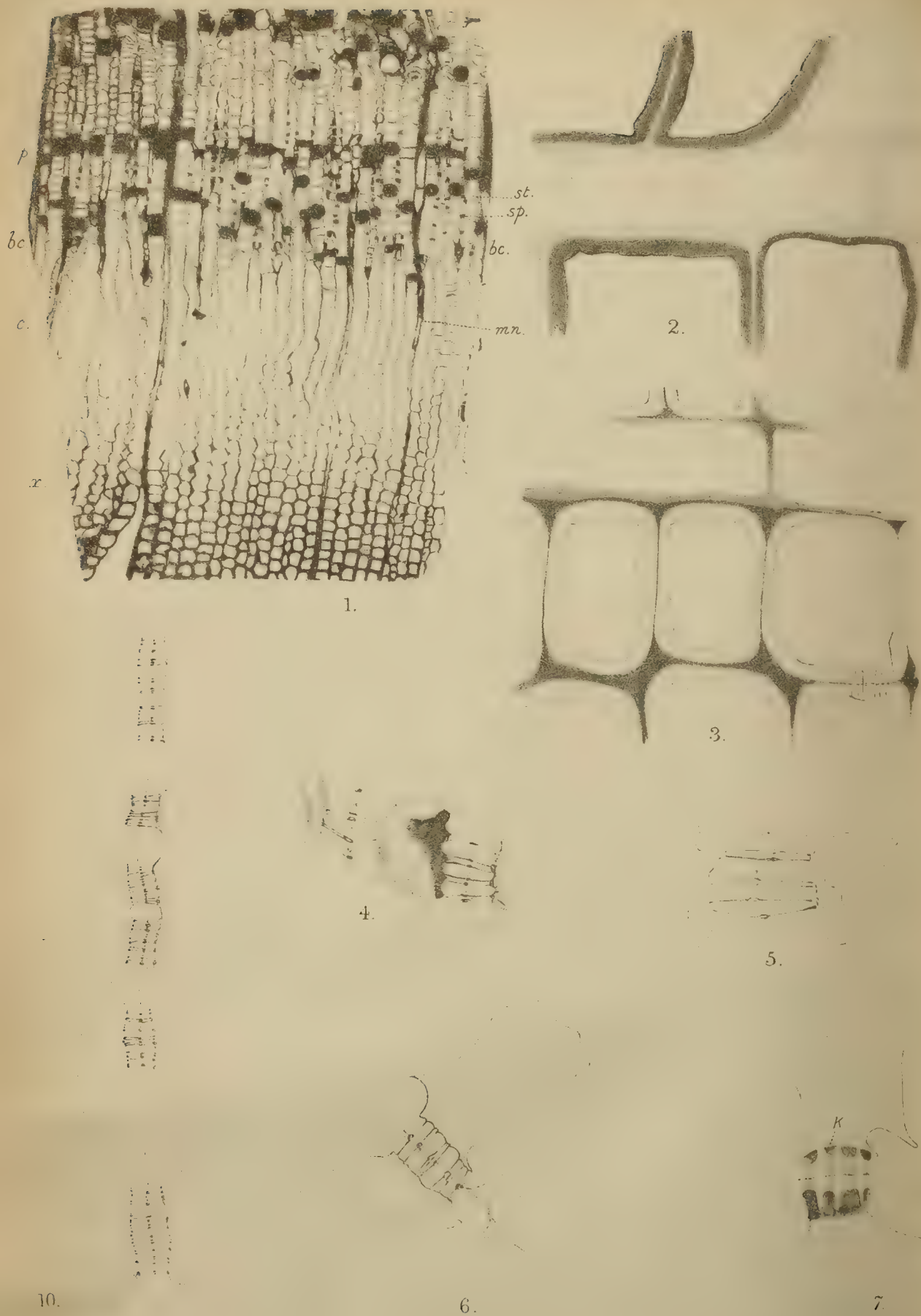
16.

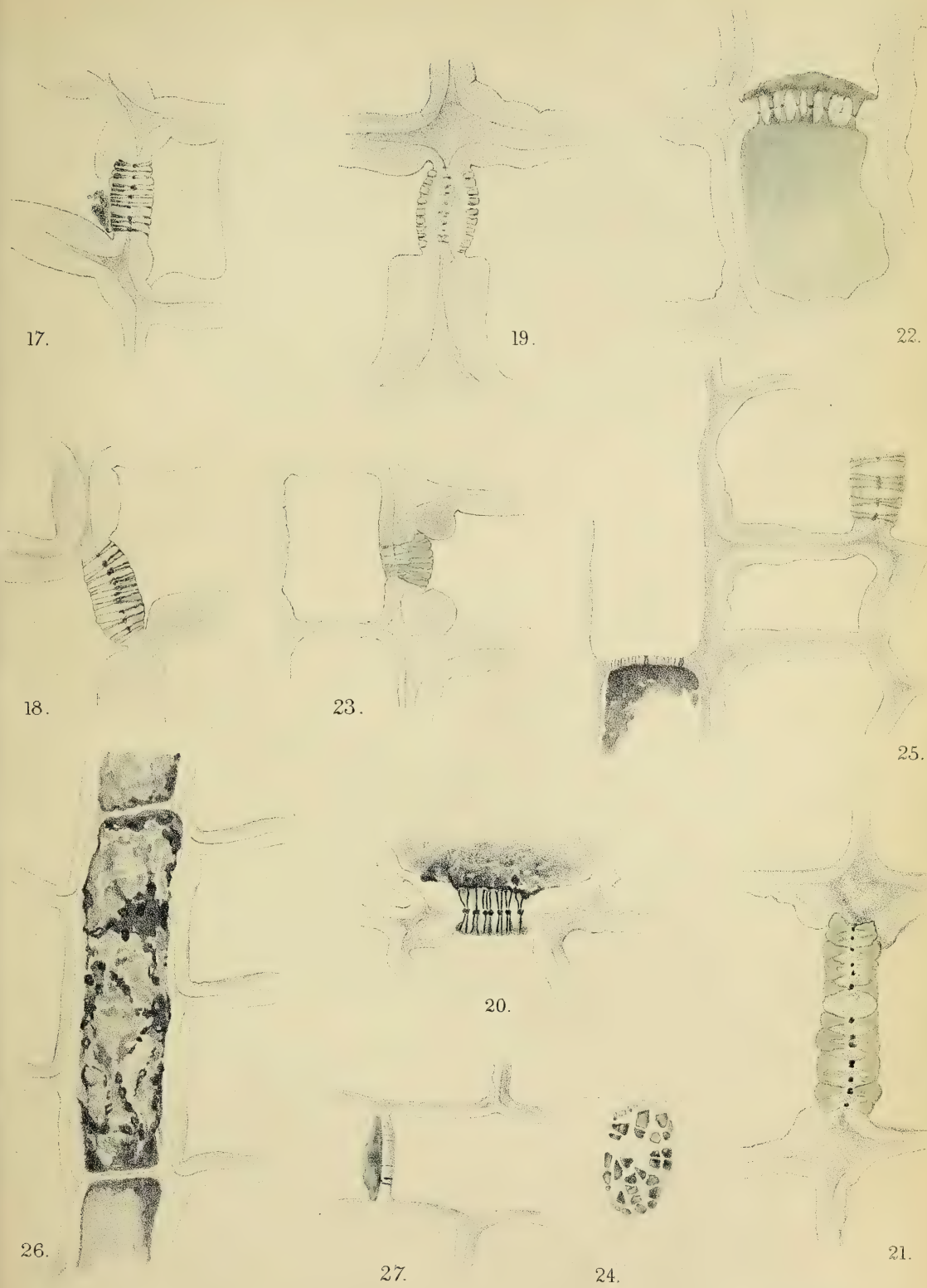


11.



14.





A. W. H. del.

University Press, Oxford.

SIEVE-TUBES OF PINUS.

On Correlation in the Growth of Roots and Shoots (Second Paper)¹.

BY

L. KNY.

ON the occasion of the Meeting of the British Association for the Advancement of Science at Oxford, in 1894, I read a paper² before Section D on the subject indicated by the above title. My investigations had led me to the conclusion, that in seedlings of the species under operation (*Vicia Faba* and *Zea Mays*), root and shoot are to a high degree independent of each other in their growth, but that, on the other hand, in the case of willow-cuttings the effect of the repeated removal of the one organ soon became apparent in the diminution of the other.

Two years later Hering published a paper in the 'Jahrbücher für wissensch. Botanik'³ on the same subject, but only with reference to seedlings. Whilst confirming my results, he regards it as unfortunate, that I determined only the final result of experiments extending over considerable periods

¹ Read before the Botanical Section of the British Association at the Glasgow Meeting, Sept. 1901.

² *Annals of Botany*, viii, p. 265.

³ *Ibid.* xxix, p. 132, 1896.

of time, and not the detailed steps by which the final result was brought about. The independence in growth of root and shoot is not, he thinks, found to be so complete, if the growth of the root be investigated immediately after the removing of the shoot. The author refers to some unpublished investigations by Stone, made in the Botanical Laboratory of the University of Leipzig, who observed at short intervals the growth of, for instance, the root of a *Vicia*-seedling, at first under normal conditions, the seedling not having been deprived of any of its parts; the observations were made by means of a horizontal microscope provided with a micrometer. The increments of growth were expressed in the form of a curve, and showed the normal course. After a period of observation under these conditions, Stone cut off the shoot of the seedling. The effect of this at once became evident in a retardation of the rate of growth of the root, the curve rising less steeply. The time required for the manifestation of this reaction naturally varied in different cases, as did also the period of the retarded growth. Retardation was followed by vigorous growth connected with the reparatory activity at the wounded surface where the shoot had been removed. Indeed, the accelerated growth of the root was sometimes such, that it not only regained the whole of the retardation, but became more rapid than that in a normal, uninjured seedling.

In all cases removal of the shoot caused temporary retardation in the rate of growth of the root. These observations, according to Hering, show that so high a degree of independence in growth as was asserted by Kny, on the evidence of the ultimate phenomena of growth, does not exist, although it is possible for the seedling to develop the shoot without a root-system, and *vice versa*, to a considerable extent, so long as there is a sufficient supply of reserve material.

It is clear that Hering had not considered the question as to whether or not the retarded growth of the root following upon the removal of the shoot was the result of the injury temporarily disturbing the development of the organism, and

quite independent of any correlation. It was just because I thought that this was probably the case, that I purposely omitted from consideration the variations in growth which immediately followed the injury. How necessary this precaution was is shown by the researches of Townsend¹, who arrived at the following conclusions² as the result of careful study:—

‘A single irritation produced by cutting or splitting the shoots or roots or removing the leaf-tips of seedlings tends to produce a change in the rate of growth of the injured and of the uninjured parts.

‘If the injury is slight, signs of an acceleration in the rate of growth will be apparent in from six to twenty-four hours and will continue for from one to several days. If the injury is severe, the acceleration will be preceded by a period of retardation, depending upon the severity of the injury and upon the condition of the plant injured.

‘The growth of the stems of older plants is accelerated by removal of a number of the roots or leaves, but is not affected by a slight injury to the roots.

‘The roots of older plants as well as of seedlings are more independent than are the stems or shoots³.

‘The change in the rate of growth of higher plants under the influence of a single irritation begins gradually, reaches its maximum in from twelve to ninety-six hours, and gradually diminishes until the normal rate is resumed.’

The results which Hering obtained by the application of the method of embedding in gypsum⁴, introduced by Pfeffer, mark

¹ The Correlation of Growth under the Influence of Injuries; *Ann. Bot.*, xi, p. 509 ff., 1897.

² *Loc. cit.*, p. 531.

³ ‘Kny, *loc. cit.*, p. 280.’

⁴ Although the method of embedding in gypsum is preferable in the present case to that of the removal of the organs, yet it must not be forgotten that the green shoot, when embedded in gypsum, is not only hindered in its growth, but is withdrawn from the influence of light, and is consequently unable to assimilate carbon dioxide. It is therefore necessary for the purpose of comparing normal plants with those embedded in gypsum, that all the experiments should be performed in darkness (*cf. Hering, loc. cit.*, p. 155). But results obtained by experiments in darkness are not directly applicable to seedlings observed in light.

an advance in the investigation of the problem under consideration. He observed in all cases an evident retardation of growth in those parts of the organism which remained free, whilst the others were imbedded in gypsum. If the shoot were embedded, the root grew more slowly than that of a free plant, and *vice versa*¹. If the parts which had been embedded were freed as carefully as possible from the gypsum, then the corresponding root and shoot at once began to grow more quickly². Hence the striking independence in the growth of roots and shoots of seedlings which was so unmistakably shown in my experiments would appear to be, *at least in part*, a result of the stimulus (*Reiz*) exerted upon the growth of the root by the regeneration of the shoot, and *vice versa*. But that this is not the only cause of the independence is clear from the observations on willow-cuttings which I have published. For although in the case of the cuttings, as in that of the seedlings, the developing shoots and roots were repeatedly removed, their independence of growth was much less marked. The experiments performed in 1894 with cuttings of *Salix acuminata* and *S. purpurea* were repeated by me in a similar manner in the following year with cuttings of *Ampelopsis quinquefolia*. In this case also, sixty-three cuttings about 27 cm. in length were arranged in nine cylinders, all of the same cubic contents. The lower ends of the cuttings were immersed in water, whilst the upper ends were in the air. Three of the cylinders formed one series. The cuttings, whose diameter varied from 6 to 11.5 mm., were so distributed, that each series included the same number of cuttings of each different diameter.

In Series I the cuttings remained uninjured throughout the experiment. In Series II developing buds were repeatedly removed with care, whilst the roots were not touched. In Series III the developing roots were repeatedly and carefully removed, whilst the buds were left untouched.

The experiments began on April 5, 1895. The operations

¹ Loc. cit., p. 139.

² Loc. cit., p. 142.

in which the developing shoots in Series II and the developing roots in Series III were removed with a pointed pair of scissors, the water being usually changed at the same time, took place on April 23 and 29, May 4, 6, 10, 16, 20, 22, 25, and 27.

The cultures flourished well, although some masses of Bacteria and some little tufts of *Saprolegnia* had formed upon the surface of the bark. The cuttings first formed a wound-callus at their organically upper ends, which had been covered with wax. Callous outgrowths were also formed at the lenticels.

On May 16 I first noticed that the shoots of Series III, where the roots had been repeatedly removed, were less vigorously developed as compared with those of Series I. This was more conspicuous on May 20, when the leaf-blades of Series I were found to be, on the average, distinctly longer than those of Series III.

In Series II, where the shoots had been repeatedly removed, a slight inferiority of development of the roots, as compared with those of Series I, could first be detected on May 20. In both series the older roots already bore lateral roots.

On May 25, both the primary and secondary roots of Series I were distinctly of greater average length than those of Series II. Also the shoots of Series I were of greater average length and bore larger leaves than those of Series III.

On May 31, when the experiment terminated, all the sixty-three cuttings were still quite healthy. Investigation showed that they all still contained abundant stores of starch in all parts. The difference in the external features of the three series had become more marked. In Series III only one shoot had attained any considerable length, whilst in Series I four shoots had largely grown out, and the leaf-blades of Series I were of greater average area than were those of Series III. The difference between the roots of Series I and II was clearly in favour of the former, though it was less striking than in the case of the shoots.

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The following are the numerical results of the measurements:—

Series I. Uninjured cuttings:—

Length of the longest root	276 mm.
Total fresh weight of roots	20.26 gr.
„ dry „ „	1.024 gr.
Length of the longest shoot	408 mm.
Total fresh weight of shoots	92.1 gr.
„ dry „ „	12.775 gr.
Length of the longest leaf-segment .	129 mm.

Series II. Buds repeatedly removed:—

Length of the longest root	269 mm.
Total fresh weight of roots	9.55 gr.
„ dry „ „	0.598 gr.

Series III. Roots repeatedly removed:—

Length of the longest shoot	321 mm.
Total fresh weight of shoots	52.1 gr.
„ dry „ „	8.607 gr.
Length of the longest leaf-segment .	110 mm.

(N.B. Only one leaf of Series III had a segment of this length. In all the others they were much shorter.)

From what has been stated above, it follows that in cuttings of *Ampelopsis quinquefolia*, just as in those of *Salix acuminata* and *S. purpurea*, the continual removal of the young shoots was soon followed by a less vigorous development of roots, and *vice versa*. There is, however, this difference to be noted, that whereas in *Salix* the retarding influence is to be detected first in the roots, in *Ampelopsis* it is the shoots which in this case proved themselves more sensitive than the roots.

Starch-Formation in *Hydrodictyon utriculatum*.

BY

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With Plate XXXIV.

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WHILE the subject of starch-formation in the plant-cell has received a great deal of attention from botanists from the microchemical standpoint, very little has been done in the way of following, by the aid of suitable technique, the sequence of structural changes undergone by the cell in such a process. This is particularly true in the case of the green Algae where the formation of the starch is connected with the pyrenoid. The cells of *Hydrodictyon* furnish material suitable for working out the sequence of events in the manufacture of starch, and I have been able to determine in them a number of facts, heretofore undescribed, which help to explain some of the conflicting statements of previous investigators.

The observations of Schmitz¹, Meyer², and Schimper³ as

¹ Die Chromatophoren der Algen, 1882: Beiträge zur Kenntniss der Chromatophoren. Jahrb. f. wiss. Bot., Bd. xv, 1884, p. 1.

² Ueber Krystalloide der Trophoplasten und über die Chromoplasten der Angiospermen. Botanische Zeitung, Bd. xli, 1883, pp. 489, 505, 525.

³ Ueber die Entwicklung der Chlorophyllkörner und Farbkörper. Bot. Ztg., Bd. xli, 1883, pp. 105, 121, 137, 153, 809. Untersuchungen über die Chlorophyllkörper und die ihnen homologen Gebilde. Jahrb. f. wiss. Bot., Bd. xvi, p. 247.

to the nature and function of the pyrenoid are too well known to need more than a very brief review. Schmitz, to whom we are indebted for the first authentic description of the pyrenoid itself, described it as a spherical proteid body, forming a part of the chromatophore and bearing a morphological relation to the latter similar to that of the nucleole to the nucleus.

Chemically, the substance of the pyrenoid was considered to be similar to the chromatin of the nucleus. But the evidence for this view of the chemical nature of the pyrenoid was, as Schimper¹ afterwards pointed out, entirely insufficient. Schmitz believed that there was a genetic relation between the pyrenoid and starch in that the pyrenoid substance was transformed into starch, the latter, however, being laid down in the substance of the chromatophore, and not in the substance of the pyrenoid. But in addition to the starch thus formed around the pyrenoid, he described its formation in other parts of the chromatophore as well. According to Schmitz there are two types of starch in the chromatophores with pyrenoids, viz. that formed in connexion with the pyrenoid and that formed in other parts of the chromatophore without reference to the pyrenoid.

Meyer insisted upon the crystalloidal nature of the pyrenoid and that it has no special function beyond that of being reserve proteid material. He observed cases in which the pyrenoid was angular rather than spherical in outline.

Schimper agrees with Meyer in the view of the crystalloidal nature of the pyrenoid, but thinks that it has a function in the formation of starch, as Schmitz had maintained previously. But neither Schmitz nor Schimper were able to bring any direct evidence for this conclusion beyond the fact that, as they thought, part of the starch is formed in the immediate vicinity of the pyrenoid.

More recently, Chmielewski² has described a star-shaped

¹ Jahrb. f. wiss. Bot., Bd. xvi, p. 247.

² Ueber Bau u. Vermehrung der Pyrenoide bei einigen Algen. Ref., Bot.

pyrenoid in *Spirogyra* and *Zygnema* whose points project between the parts of the surrounding starch-layer. Chmielewski insists that the pyrenoid is a permanent cell-organ always arising by the division of a pre-existing pyrenoid, a view previously suggested by Schmitz and Strasburger¹; still Schmitz also described the origin of pyrenoids in *Nemalion*, *de novo*—and Strasburger described the disappearance of the pyrenoids prior to the formation of the swarm-spores in *Cladophora*.

In his paper on the formation of the reproductive cells of *Hydrodictyon* Klebs² attempted to establish, by certain experimental culture-methods, a difference between the so-called pyrenoid-starch and the stroma-starch. But in this work Klebs, like his predecessors, failed to find the real genetic relation between the pyrenoid and all of the starch.

From the brief *résumé* given above it may readily be seen that there has been very little contributed towards the solution of the problem as to the nature of the relation of the pyrenoid to the formation of starch. This has been apparently due to two causes, the failure to find stages showing active starch-formation, and inadequate methods of treatment in studying the early and most important stages in the process.

The material I have used was fixed in either Merkel's fluid or in one of the two iridium chloride and acetic acid mixtures whose formulas I have already published in another connexion. The material was embedded in paraffin and cut on a microtome into sections of from three to six μ in thickness. The triple stain of Flemming gives a very clear differentiation, staining the pyrenoid bright red and the starch blue, while the surrounding cytoplasm is stained orange³.

The general structure of the *Hydrodictyon* cell has long

Centralbl., Bd. lxi, p. 277. Die Pyrenoide. Bot. Centralbl., Bd. lxxvii, p. 108.

¹ Zellbildung u. Zelltheilung.

² Fortpflanzungszellen bei *Hydrodictyon utriculatum*, Roth. Bot. Ztg., 1891.

³ A more detailed account of the method of procuring and treating the material will be published later, in another connexion.

been known to be that of a cylindrical coenocyte, having on the outside a thick cell-wall, and within this a layer of protoplasm surrounding a large central vacuole. Seen in section the protoplasm is clearly bounded on the outside by a plasma-membrane and on the inside by a vacuolar membrane of similar appearance. Between these two membranes the protoplasm contains the nuclei and pyrenoids and vacuoles of various size. There is no indication of any differentiation into a layer containing the chlorophyll and one containing nuclei. *Hydrodictyon* contains no differentiated chromatophore. In *Chlamydomonas* Dangeard has recently sought to establish the presence of a distinct chromatophore by the fact that the protoplasm of the chromatophore shows an alveolar structure, while the surrounding cytoplasm is more like a network. An examination of Figs. 21 and 26 (Pl. XXXIV) will show clearly that no such differentiation can be made out in *Hydrodictyon*. The cytoplasm shows no differentiation, such as is always observed in *Spirogyra* cells fixed and stained in identically the same way. This agrees with what is observed in the living cells of *Hydrodictyon*, in which, in spite of the statement of Klebs and Artari, it is plain that the chlorophyll is distributed in the whole peripheral protoplasmic layer of the cell. Most striking evidence of the lack of a distinct chromatophore is also to be found in the distribution of the pyrenoids and nuclei. The pyrenoid, as Schmitz pointed out, belongs to that part of the protoplasm that contains the chlorophyll, i. e. to the chromatophore where it exists as a distinct part of the cell. If, then, there is a distinct chromatophore here we should expect to find its position indicated by the position of the pyrenoids. On the other hand, the nuclei would lie in a layer outside or inside the chromatophore. That this is not the case is shown clearly in figures where both pyrenoids and nuclei are seen to be scattered throughout the protoplasmic layer, neither being confined to any one region (Figs. 21 and 26). Moreover, the two bodies are often in immediate juxtaposition so that it is impossible that one should be considered as in the chromato-

phore and the other not (Fig. 27). Still another point of importance is the fact that in those cells containing an abundance of starch, practically the whole layer of protoplasm, from the plasma-membrane on the outside to the vacuolar membrane on the inside, is filled with the starch-grains, all of which, as I shall show below, have their origin in the pyrenoid, and are later transferred bodily to other parts of the cell (Fig. 26). If we are to think of a differentiated chromatophore we should expect the starch to be retained within its body, as is clearly the case, for example, in *Spirogyra*. It would seem that, then, *Hydrodictyon* is to be placed among those plants in which the chlorophyll is distributed generally in the cytoplasmic part of the protoplast. Klebs and Artari¹ have described a chromatophore in *Hydrodictyon* made up of a complicated network, which may, according to Artari, become a single slightly alveolar layer, or, according to Klebs, become divided into several layers having a net-like structure. Both of these observers worked, however, with surface-views of the cells, a condition under which accurate observation of the whole protoplast is quite impossible. The numerous vacuoles which frequently appear in the cells might easily lead to some such interpretation as that of Klebs or Artari, provided only surface-views were had.

It is an interesting question whether the condition above described for *Hydrodictyon* is a primitive condition in chromatophore-development. Prior to the appearance of Schmitz's work on the chromatophores of the Algae, it was not uncommon to have forms described as having the chlorophyll diffused throughout the cytoplasm. Schmitz first insisted upon the doctrine that the chromatophore in all Algae was a distinct cell-organ, and that there was no such thing as chlorophyll contained in undifferentiated cytoplasm. This doctrine of Schmitz was soon extended to all of the so-called plastids by Schimper, and is now the prevailing theory among botanists. Still it is not impossible that a more careful investigation of a large number of forms will reveal conditions

¹ Zur Entwicklungsgeschichte des Wassernetzes. Moskau, 1890.

in many like those in *Hydrodictyon*. It may not be without significance that whereas there is no differentiated body here which can be distinguished as a chromatophore, still there are well-defined centres (the pyrenoids) for the formation of starch.

If we turn now to the details of starch-formation it is to be noted that the whole process can be traced from certain structural changes occurring in the body of the pyrenoid. This body, while it is not undergoing such transformations, presents a deep red homogeneous appearance in sections treated with the safranin gentian-orange mixture. In outline it may present many variations in form depending largely upon its relation to the process of starch-formation as described below. The typical appearance, however, of the resting pyrenoid is, perhaps, that described by Schmitz, in which the pyrenoid shows a spherical form with a clear outline. In this condition it is often surrounded by a single layer of starch made of separate grains of a decidedly concavo-convex form (Fig. 21). This is a condition that is very often observed and forms the basis of most of the familiar descriptions of the pyrenoid. That it is a resting condition, however, is abundantly shown by the fact that during the formation of starch a complete series of stages may be found, leading from it to the conditions in which starch-formation seems to be most active, and by the further fact that it generally appears prior to the formation of swarm-spores, when starch-formation has ceased and the processes leading to the disappearance of both starch and pyrenoids have set in. The first indication of the changes leading to the formation of starch consists of a differentiation of the body of the pyrenoid into two portions, one of which is destined to become transformed into a starch-grain and the other to remain unchanged. The part that is to form the starch soon stains less densely. Instead of being stained red it now becomes a neutral grey, or faint orange. The portion so differentiated may include nearly half of the pyrenoid, but frequently does not include so much (Figs. 1-4 and 6-8). In connexion with the change in staining capacity,

there are some important structural changes to be noted. The dense homogeneous structure becomes more spongy, with denser and less dense regions. There is, however, no regularity in the form or distribution of such regions. Frequently there is simply a dense region toward the outside with the less dense toward the unchanged part of the pyrenoid (Figs. 3 and 8). Very often, as indicated above, the denser regions may be so distributed as to give the appearance of a roughly alveolar structure (Fig. 6). In any case the denser regions soon become more prominent and take up the blue stain. They apparently increase in size until nearly the whole part of the pyrenoid undergoing transformation presents a deeply blue stained homogeneous structure, the typical appearance of all the starch-grains in this plant. There is nearly always between the fully formed starch-grain and the unchanged remainder of the pyrenoid a thin zone of slightly stained material (Figs. 1 and 6), as if with increasing density there were a shrinkage in the whole mass. Such a zone may appear before the starch-grain has reached the homogeneous character of the mature grain. There is in effect a splitting off of a segment of the pyrenoid which is metamorphized into a starch-grain. When the grain of starch is fully formed it appears to lie in a vesicle or vacuole in the cytoplasm, but without being surrounded by a differentiated membrane. The vesicular appearance may of course be due to a slight contraction of the protoplasm in fixing, but this is a point that could not be settled definitely.

The mature grain has practically the shape of the segment of the pyrenoid from which it is formed. Even after it is widely separated from the pyrenoid, in the manner described below, the plano-convex or concavo-convex form is retained (Fig. 1). This is a point of much importance, in establishing the fact that all of the starch is formed from the pyrenoid.

When starch is being formed rapidly a second grain will be built at once before the pyrenoid regains its original form. The remaining part of the pyrenoid undergoes the same differentiation as was seen in the original body, and the

differentiation occurs in such a fashion that the long axis of the second grain is at right angles to that of the first: that is, a second segment is cut off from the pyrenoid with the plane of cleavage at right angles to that in which the first grain was cut off. This rule is not invariable, but I have never, for example, found a second grain formed between the first and the remainder of the pyrenoid. Of course it is impossible to establish, with any certainty, the sequence in which any great series of grains have arisen such as are shown in Figs. 1-4; but the general arrangement would seem to support the above statement. So far as the pyrenoid itself is concerned it is hard to understand why a second grain should not follow the first on the same side. Still the process as followed here is better adapted to secure an equal distribution of the starch through the cytoplasm, and this fact may have led to the establishment of a more or less fixed order in the formation of the grains as described above.

It is possible, too, that the formation of the starch depends upon the action of the surrounding cytoplasm as well as of the pyrenoid itself. In this case when a starch-grain is fully formed it would separate the two and thus, perhaps, lead to a shifting in the place of formation for the next grain.

When the process of starch-formation is going on very rapidly, the grains, as they are formed, are continually crowded outward by the later-formed grains, so that finally, as previously indicated, they are densely packed through nearly the whole protoplast (Figs. 1 and 26). This condition is undoubtedly what led to the distinction made by Schmitz, and later worked out in more detail by Klebs, between starch formed around the pyrenoid (pyrenoid-starch) and that formed in other parts of the chromatophore (stroma-starch). But that there are not two kinds of starch, at least in their origin, is made clear by an inspection of Figs. 1-5. It is to be noted in these cases that no distinction whatever can be made between the starch immediately surrounding the pyrenoid and that farther out in the cytoplasm. Moreover, the form and arrangement of the whole mass of starch-grains, as shown

in Fig. 1, is seen to have in a general way, but quite clearly, orientation about the pyrenoid as a centre. The concentric arrangement will be especially noted in Fig. 5. This arrangement seems to point clearly to the common origin of all the starch-grains from the pyrenoid in the manner described above.

The failure on the part of former observers to trace the origin of all the starch to the pyrenoid is due, as I have previously indicated, to the lack of material taken in a condition of active starch-development, coupled with methods of treatment inadequate to bring out the stages in the genesis of the individual grains. That various observers have drawn their conclusions from a study of isolated stages in starch-development is shown by the conflicting statements as to the form of the pyrenoid itself. Schmitz maintained that it is a spherical body, a conclusion doubtless drawn from a study of resting pyrenoids as described above. Meyer probably saw a single stage in starch-formation and was led to insist upon the angular form of the pyrenoid. It is even possible that the star-shaped pyrenoid described by Chmielewski was simply a condition similar to that shown in Fig. 4.

The size of the pyrenoids at the time they are forming or about to form starch is very variable. While in many cases the pyrenoids are of approximately the same size in any one cell during active starch-formation (Fig. 1), in many others there is a very wide variation. Two pyrenoids representing almost extremes in size are frequently found lying side by side in the same part of the cell, either or both of which may show the beginning of starch-formation (Fig. 21). Whether these represent pyrenoids of different ages with independent genetic histories, or whether they are sister organs formed as the result of the division of a pre-existing pyrenoid, is an important question, as yet unsettled. There is abundant evidence that the development of the starch may take place in such a way as to completely divide the pyrenoid into two parts. This is most often brought about by the equatorial region of the pyrenoid being transformed into starch in such

a fashion as to leave an undifferentiated portion on either side of the starch-grain (Figs. 10 and 15). In the latter figure the constricted appearance of the pyrenoid in the region of starch-formation is noteworthy. The explanation of this fact is not evident, but it may be due simply to the shape of the grains earlier formed, an hypothesis that seems to be supported by the shape of the grains on either side of the pyrenoid. Fig. 11 is quite striking in this respect, while Fig. 12 shows no such clear relation of the shape of the starch-grains to that of the pyrenoids. Note also that in Figs. 11 and 12 there is no evidence of starch-formation in the constricted region.

Whether the pair of pyrenoids in the central part of Fig. 6 is the result of the division of a single pyrenoid is not clear. The peculiar shape of the starch-grain lying between the two pyrenoids is uncommon, and may possibly be the result of a fusion of the parts of two closely adjacent pyrenoids that are being metamorphized into starch. But earlier stages indicating such a fusion have not been observed, and it is not impossible that the starch-grain simply represents a middle portion of the original single pyrenoid after it had the constricted appearance, as a result of the formation of the older grains on either side.

It is, I think, sufficiently clear that there may be a division of the pyrenoid due to a transformation of a certain part of it into starch. Whether all the cases of division of the pyrenoid that have been described by other observers are to be explained in this manner is an interesting question that needs further careful investigation. The apparent time-relations of the division of the pyrenoids in *Spirogyra* and *Zygnema* (in the one case immediately preceding and in the other immediately following the division of the cell), as described by Strasburger and Chmielewski, would seem to have little relation to the process as described above for *Hydrodictyon*. Still, a single pyrenoid in the condition shown in Figs. 10 or 18 might well suggest, if taken alone, a reproduction of pyrenoids rather than starch-formation. That the pyrenoids

of *Hydrodictyon* disappear prior to the formation of the swarm-spores or gametes has been known since Braun's classic researches on Rejuvenescence in Nature. This of course necessitates an origin *de novo* in the young cells. Hence division cannot at least be considered the only means for the multiplication of pyrenoids.

The steps in the disappearance of the pyrenoids and usually of the starch prior to cleavage are worthy of attention, and for the starch may be taken as typical for the process of starch-solution in the plant. Generally the first starch to disappear is, as might be expected, that farthest from the pyrenoids. The starch-grains seem to be dissolved uniformly over the surface instead of in furrows and by irregular corrosions as is often observed in the storage-starch of the higher plants. Very often a stage is seen where there remains but a single layer of starch around the pyrenoid, while the pyrenoid itself shows no indication of further starch-formation, but has the homogeneous structure and spherical form of the typical resting pyrenoid (Fig. 21: large pyrenoid). This stage is also often met with in cells that give no other indications of reproductive activity. It probably represents, as previously suggested, a quiescent condition of the cell so far as starch-formation is concerned, and the frequency of its appearance serves to explain Schmitz's account of the pyrenoid as a spherical body surrounded by a single layer of starch. Such stages must be carefully distinguished from that represented in Fig. 6, where there is manifestly development of starch taking place, but where also the solution of the starch seems to wellnigh keep pace with its formation so that there is but little more than a single layer of starch around the pyrenoid. Let the process of starch-formation cease in such a condition as this last, and we can very readily imagine the whole structure passing over into such a resting-stage as that shown in Fig. 21. As the solution of starch continues, the pyrenoid itself generally becomes smaller, although the starch is usually all dissolved before the pyrenoid has entirely disappeared (Figs. 22-24). Still, it is not necessary that either

the starch or the pyrenoid should wholly disappear before the spores are formed; for, as Klebs observed, and as many of my preparations show, in spores just formed, as well as during cleavage-stages, an abundance of starch may be seen, always appearing as if in a stage of solution (Fig. 27). Pyrenoids may also be present at these stages. Klebs thought the starch to be what he called the stroma-starch, as he failed to see the remaining pyrenoids here and there through the segmenting protoplasm, and even in some of the swarm-spores (Fig. 28). In such pyrenoids, however, I have never seen any indication of starch-formation. My preparations seem to show that the formation of starch has ceased, but that conditions were such that cleavage took place without the preliminary solution of the starch as well as the body of the pyrenoids. The relation of the pyrenoids and starch to spore-formation I hope to discuss more fully in connexion with an account, now in preparation, of the spore-formation. It should be especially noted that the presence of starch-grains in the young spores that contain no pyrenoids does not mean the formation of such grains *in situ*, but that they are grains of starch formed in the manner previously described, and simply remaining in the portion of the protoplasm forming the spore. In this connexion it may be further noted that under certain conditions the pyrenoid itself may entirely disappear, leaving the surrounding starch-layer or layers still intact (Figs. 19 and 20). This condition seems to arise in cells in which there is no sign of cleavage to be found, but it is possible that it may also occur just prior to cleavage, so that all the swarm-spores formed in any one cell may contain starch, but none of them show a pyrenoid. It should be borne in mind that, as Klebs has pointed out, prior to cleavage the number of nuclei in the mother-cell is far in excess of the number of pyrenoids, so that even should the pyrenoids persist during cleavage, as they in some cases do, many of the uninucleate spores would be without them, a fact that seems to point unmistakably to the *de novo* origin of pyrenoids in the young cells.

During the solution of starch around the pyrenoids there is

frequently an appearance indicative of the formation of a vesicle containing the dissolved substance of the grain. This presents often the appearance of a homogeneous layer, having a faint blue or grey colour surrounding the whole pyrenoid (Figs. 18 and 27). In it may often be seen portions of the partly dissolved starch-grains (Figs. 16 and 17). The significance of such a condition is not entirely clear, but it seems to suggest that the product of the solution of the starch may not be used as fast as formed. On the other hand, cases frequently occur where the grains, being dissolved, seem to lie imbedded in the protoplasm without any surrounding vesicle, and the pyrenoid itself, where the starch is entirely dissolved, is in immediate contact with the protoplasm (Fig. 25).

The condition just described, in which the products of the solution of the starch appear in a vesicle surrounding the pyrenoid, is probably quite distinct from that shown in Figs. 7, 8, and 9, where there is a clear space surrounding the young pyrenoids. Boubier's¹ recent attempt to establish the region of starch-formation in this space, including it in the pyrenoid, is entirely unsupported so far as the facts in *Hydrodictyon* go. In no case have I found either the net-like structure or the delimiting, distinct membrane that Boubier described. The outer sharp boundary of the vesicle in these stages is not to be taken as a permanent membrane, for, besides being entirely wanting in many cases where there is no starch present, it is also lacking in every case of rapid starch-formation.

CONCLUSIONS.

If we turn now to a brief consideration of the bearing of the facts described in the foregoing pages upon the general processes of metabolism in the cell, it is of most importance to note that the pyrenoid is directly the seat of the processes resulting in the formation of starch. Both the structural and microchemical changes shown by differences in staining indicate that the process is an exceedingly complicated one,

¹ La membrane pyrénoidienne; Bull. de l'Herbier Boissier, tome vii, p. 451.

involving many chemical transformations whose nature must be left to future investigations to determine. The fact that, as seems to be well established by various observers, the pyrenoid is of a proteid nature, and that a portion of it—at least in *Hydrodictyon*—is converted into starch, suggests that the process involves the breaking down of a proteid into a carbohydrate. Still, it is not to be thought that the substance of the pyrenoid is merely passive in the process, for on that assumption it would be hard to explain the differentiation of its body into two portions, one of which is transformed into starch, while the other retains the original pyrenoid character.

To be sure the pyrenoids themselves may totally disappear at certain periods in the life-history of the cell, notably prior to reproduction; but it is quite probable that active cell-organs may disappear when their work is not immediately needed, and be formed anew when their activities become necessary. (Cf. Wilson, *The Cell*, p. 305, as to the centrosome.)

Whether the pyrenoid represents an active, independent cell-organ whose function is the formation of starch, or whether it is to be thought of as a mere stage in the process of starch-formation whose real seat is to be found in the chlorophyll-bearing cytoplasm, is not certain from the facts thus far at hand. As pointed out above, the method of differentiation of the body of the pyrenoid preceding the actual formation of the starch indicates that the pyrenoid is more than a passive body representing a stage in the process. The genetic history of the pyrenoid itself, including its relations to the chromatophore, where a differentiated chromatophore exists, or to the cytoplasm in such a form as *Hydrodictyon*, would perhaps throw some light on the nature of the whole metabolic processes resulting in starch-formation.

In the paper previously referred to, Boubier developed the interesting hypothesis that the pyrenoid is comparable to the leucoplast of the higher plants, and that the method of starch-formation in it is similar to that in the latter bodies. While the suggestion may later prove to be a valuable one, still

the evidence in favour of it is not sufficient to be convincing. The fact that the pyrenoid is a temporary structure does not certainly disprove the truth of the suggestion, for, as Eberdt has maintained, it is possible that the leucoplast may arise *de novo* in the cell. It is perhaps more difficult to think of a leucoplast and chloroplast as associated together in the same cell. Such an association would certainly be out of harmony with the doctrine of the genetic relations of the two organs as maintained by Schimper. The most serious objection, however, to the comparison suggested by Boubier seems to me to lie in the fact of the difference in structure between the pyrenoid and leucoplast. So far as I have been able to observe, it is by no means easy to differentiate the leucoplast from the rest of the protoplasm by the ordinary methods of cytological research, such as those used in the present investigation; and when it is differentiated, it has a granular or reticulate appearance, while the pyrenoid appears homogeneous, dense, and sharply bounded.

The method of starch-formation in the pyrenoid as I have described it has, so far as I can determine, no analogy with that in the leucoplast—at least so far as its functional aspects are concerned—with the possible exception that, as maintained by Eberdt¹ and Schimper², there is a breaking down of a proteid substance to form a carbohydrate³.

Should the conclusions of Meyer⁴ and Salter⁵, that starch-formation in the leucoplast is a process of secretion within the body of the leucoplast itself, without involving any change in its structure, prove correct, Boubier's hypothesis would be

¹ Beiträge zur Entstehungsgeschichte der Stärke; Jahrb. f. wiss. Bot., Bd. xxii, p. 293.

² Untersuchungen über das Wachsthum der Stärkekörner; Bot. Ztg., Bd. xxxix, 1881, p. 185.

³ The difference between these two authors lies not in the method of starch-formation itself, but in the nature of the body in which it is formed. Schimper, as is well known, maintained that it is a permanent cell-organ, while Eberdt thought it to be a temporary body consisting of what he called starch ground-substance, which was entirely converted into starch.

⁴ Untersuchungen über die Stärkekörner. Jena, 1895.

⁵ Jahrb. f. wiss. Bot., Bd. xxxii, 1898, p. 117.

strongly negated. I am inclined to the view that the pyrenoid is an active body, differentiated in the chlorophyll-bearing cytoplasm, which in co-operation with the latter acts as the base of the process of starch-formation. In just what the co-operation consists must remain to be determined by future investigation.

If we compare the method of starch-formation in *Hydrodictyon* as described above with starch-formation in chromatophores without pyrenoids, there is little to suggest a parallel, and the relations of the two must be regarded as uncertain for the present.

MADISON, WIS., May, 1901.

EXPLANATION OF FIGURES IN PLATE XXXIV.

Illustrating Mr. Timberlake's paper on Starch-Formation in *Hydrodictyon*.

All figures were drawn with the aid of the Abbé *camera lucida*, and with the Zeiss apochromatic objective 2 mm., num. ap. 1.30, and compensation oculars 4, 12, and 18.

Fig. 1. Part of surface-section of cell, showing condition of rapid starch formation; P. pyrenoid, S. starch. $\times 1500$.

Figs. 2, 3. Details of starch-formation in single pyrenoids. $\times 2250$.

Fig. 4. Shows peculiar angular form of pyrenoid. $\times 2250$.

Fig. 5. Pyrenoid surrounded by distinctly concentrically arranged layers of starch. $\times 2250$.

Fig. 6. Starch-formation, where solution of starch is going on nearly as rapidly as its formation.

Fig. 7. Young cell, showing two small pyrenoids in which no starch is forming, and a larger one in which a single grain of starch is being formed.

Fig. 8. Same as above.

Fig. 9 *a*. Cell, showing pyrenoid without starch, and surrounded by a colourless, clearly bounded area. Pyrenoid itself shows but a faint colour. $\times 2250$.

Fig. 9 *b*. Starch-forming in pyrenoid with colourless region as before. Small pyrenoid lying against nucleus. $\times 2250$.

Figs. 10-18. Different phases of starch-formation resulting in the division of the pyrenoid. For explanations see text. $\times 2250$.

Figs. 19, 20. Showing disappearance of pyrenoid without the starch being entirely dissolved. $\times 2250$.

Fig. 21. Vertical section, showing two pyrenoids of unequal size in the same cell. The larger pyrenoid in resting stage; smaller showing starch-formation. $\times 2250$.

Figs. 22-25. Disappearance of starch and pyrenoids prior to spore-formation. $\times 2250$.

Fig. 26. Portion of vertical section, showing distribution of nuclei, pyrenoids, and starch grains in the protoplast. $\times 600$.

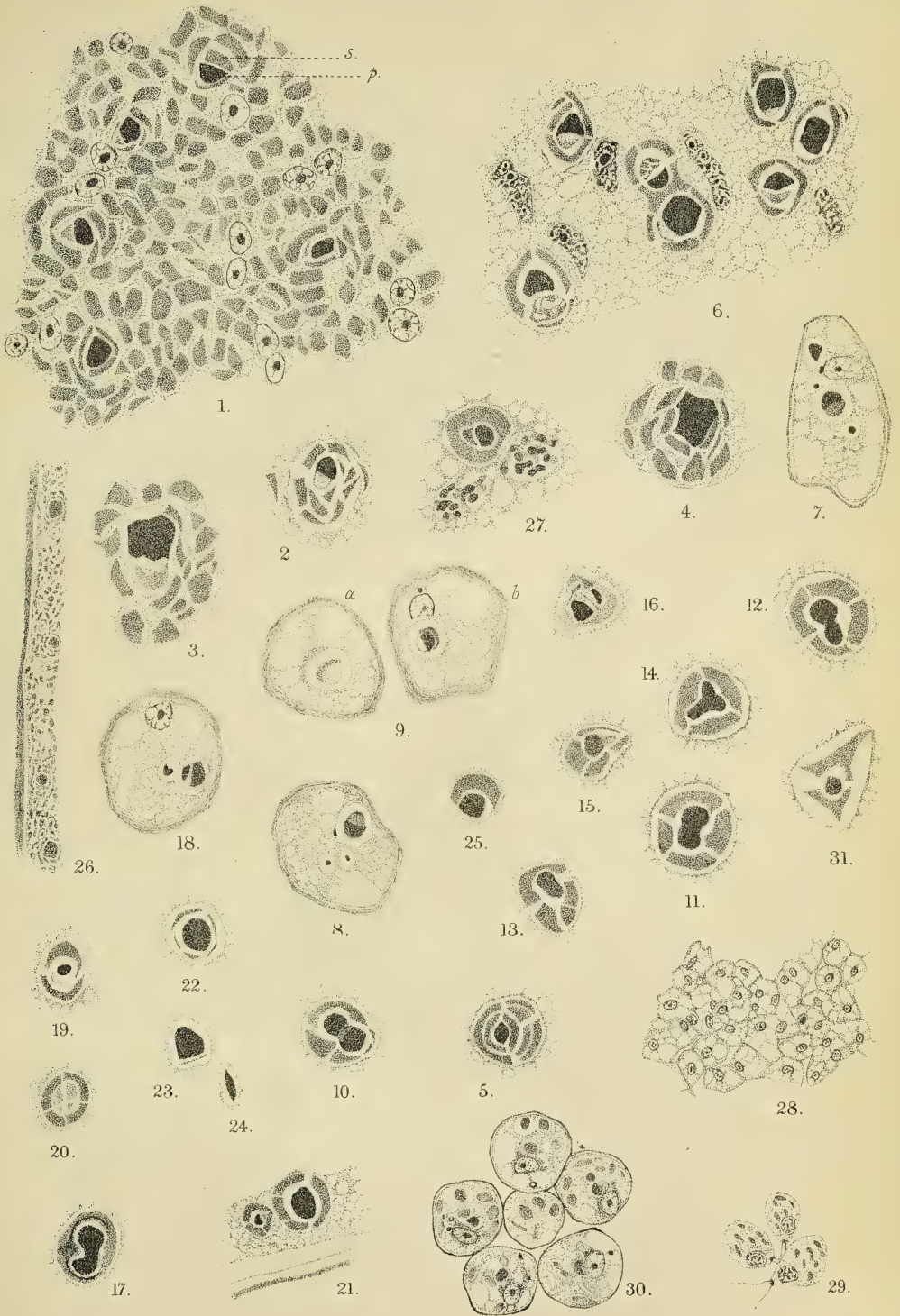
Fig. 27. A pyrenoid and two nuclei lying very close together. Nuclei are in prophases of division. Pyrenoid surrounded by homogeneous substance formed by solution of starch. $\times 2250$.

Fig. 28. Early stage of spore-formation, showing a single pyrenoid remaining in the protoplasm. $\times 600$.

Fig. 29. Young swarm-spores containing starch-grains. $\times 1500$.

Fig. 30. Young cells, each containing a pyrenoid and several starch-grains. $\times 1500$.

Fig. 31. Pyrenoid showing unusual forms of starch-grains.



The Morphology of the 'Flowers' of *Cephalotaxus*¹.

BY

W. C. WORSDELL, F.L.S.



With Plate XXXV.



I HAVE lately, through the kindness of Miss M. Benson, Lecturer in Botany at the Royal Holloway College, Egham, had placed at my disposal for investigation some abnormal female flowers of *Cephalotaxus Fortunei*, to the study of which a large part of the present memoir will be devoted.

I desire, however, to take the present favourable opportunity to enter into a general consideration of the morphology of the 'flowers' of both sexes in this important genus, for it appears to me that some uncertainty prevails with regard to the real morphological nature and relationships of the flowers. This is due in part to the fact that *Cephalotaxus* is much less frequent in gardens and parks than its ally *Taxus*, and in part to the minuteness and obscure position of the flowers.

THE STRUCTURE OF THE MALE FLOWER.

In the axils of the foliage-leaves of the previous year are borne small stalked, somewhat globose cones or capitula, the bracts of which are arranged spirally (Pl. XXXV, Fig. 1)². In the axil of each bract of the cone is placed a short axis bearing

¹ From the Jodrell Laboratory, Royal Botanic Gardens, Kew.

² This figure is taken from a photograph, for which I am indebted to Mr. L. A. Boodle, F.L.S.

[*Annals of Botany*, Vol. XV. No. LX. December, 1901.]

numerous (8–10 on the lower, 2–4 on the upper) minute appendages, the arrangement of which could not well be determined. Each of these short-stalked appendages bears in its turn two, sometimes three, pollen-sacs, more or less pendulous, or with their apical portions directed obliquely outwards and downwards, below the somewhat swollen, often pointed apex of the appendage (Pl. XXXV, Fig. 2). In fact, the structure of each axillary axis of the capitulum is precisely that of the axis both in *Ginkgo* and *Phyllocladus*, axillary to scale-leaves of the brachyblast, which bears the numerous short appendages supporting pollen-sacs to the number of two or three. In *Cephalotaxus*, however, these axillary axes are arranged in the axils of bracts, in a compact inflorescence, whereas in *Ginkgo* and *Phyllocladus* each is perfectly isolated and independent.

THE STRUCTURE OF THE FEMALE FLOWER.

The small stalked capitula arise in the axils of scale-leaves, near the base of shoots of the year. Each produces three or four pairs of bracts exhibiting the opposite-decussate arrangement on the axis, thus differing in this character from those of the male capitulum. In the axil of each bract are two erect ovules, one on either side.

HISTORICAL NOTES.

Most authors seem to be agreed as to the morphology of the male 'flowers' of the genus. Each axillary 'catkin,' as Eichler terms it, represents a single 'flower,' several of which go to constitute the capitulum which is an inflorescence. But one looks in vain in the textbooks for any definite detailed comparative treatment of the flowers, and yet this is the only sure method of determining their true morphological character. Čelakovský¹ appears to be the only botanist who has given this subject the attention which it deserves, the only one who throws full and complete illumination thereupon. I will now

¹ 'Die Gymnospermen,' &c. Abhandl. d. kgl. böhm. Ges. d. Wiss., vol. iv, 1890.

proceed to demonstrate the morphology and homologies of the flowers as indicated by him.

Undoubtedly, the nearest allies of *Cephalotaxus* are *Taxus*, *Torreya*, *Phyllocladus*, and *Ginkgo*; of these *Ginkgo* and *Phyllocladus* almost certainly stand nearest to our genus. Each axis placed axillary to a bract of a male inflorescence is the homologue of the axis in *Ginkgo* and *Phyllocladus*, standing singly and alone in the axil of a scale-leaf or foliage-leaf of the brachyblast or short shoot; this brachyblast is, therefore, probably homologous with the single inflorescence of *Cephalotaxus*, the difference from *Ginkgo* lying in the fact that in this latter genus the shoot possesses a vegetative apical bud and produces foliage- as well as scale-leaves, whereas in *Cephalotaxus* it only bears bracts. In *Taxus* and *Torreya* the male flowers also stand singly in the axils of the scale-leaves of short branches; unlike *Ginkgo* and *Cephalotaxus*, the axis of each flower of the two former genera bears several pairs of decussate bracts or sterile sporophylls; in *Phyllocladus* a single such bract is present below the sporophylls.

The short stalks which bear the pollen-sacs are sporophylls of the primitive radially-symmetrical type, but represent a slight advance on the most primitive type of all represented by the case of *Cordaite*s, in which the sacs are borne terminally on the radially-constructed sporophyll, for in *Cephalotaxus* and *Ginkgo*, *Torreya*, and *Phyllocladus* the sacs are subterminal and pendulous, owing to a slight prolongation of the axis of the sporophyll, between and beyond the sacs, into a small protuberance; this condition of things is very interesting, for it marks an intermediate, transitional stage between the most primitive type of sporophyll (as seen in *Cordaite*s, or, to take an instance from the Cycadales themselves, in *Bennettites*¹) to that of *Taxus*, where the extended terminal portion of the sporophyll has become enlarged and flattened out into a very

¹ Worsdell, 'The Affinities of the Mesozoic Fossil, *Bennettites Gibsonianus* Carr.' Ann. Bot., vol. xiv, September, 1900.

D. H. Scott, Studies in Fossil Botany, Figs. 147, 148, pp. 455, 456, 1900.

distinct peltate structure bearing the pollen-sacs (here multiplied in number) on its lower and inner surface. It is the same radially-symmetrical, peltate type of sporophyll which occurs in that primitive group the Calamariae. *Taxus* thus represents an advance from the earlier types of *Cephalotaxus*, *Ginkgo*, &c. towards the sub-peltate dorsiventral type of sporophyll of the true Coniferae. If the male 'flowers' of *Taxus* and *Ginkgo*, &c. be regarded as 'cones,' that of *Cephalotaxus* would be a compound cone: considered apart from the nature of the lateral appendages, the relationship in this respect between the two former genera and the last-named is that existing between a raceme and a panicle; in other words, the flowers of *Cephalotaxus* pertain to a higher grade of branching than those of the other genera. The relationship may be thus expressed:—

Male Flowers.

<i>Ginkgo.</i>	<i>Phyllocladus.</i>	<i>Cephalotaxus.</i>	<i>Torreya.</i>	<i>Taxus.</i>
Flowers single in axils of scale- or foliage-leaves.	Flowers single in axils of scale- or foliage-leaves.	Flowers grouped in compact inflorescence in axils of foliage-leaves.	Flowers single in axils of foliage-leaves.	Flowers single in axils of foliage-leaves.
Floral axis with no bracts below the sporophylls.	Floral axis with a single bract below the sporophylls.	Floral axis with no bracts below sporophylls.	Floral axis with bracts below the sporophylls.	Floral axis with bracts below the sporophylls.
Sporophylls radially-symmetrical in structure.	Sporophylls radially-symmetrical in structure.	Sporophylls radially-symmetrical in structure.	Sporophylls radially-symmetrical in structure.	Sporophylls radially-symmetrical in structure: peltate.
Pollen-sacs subterminal, pendulous.	Pollen - sacs subterminal, pendulous.	Pollen - sacs subterminal, pendulous.	Pollen - sacs subterminal, pendulous.	Pollen-sacs inserted on inner surface of peltate portion.

As regards the female flowers, Eichler¹ held the short, secondary shoots of *Taxus* and *Torreya*, which bear each

¹ Eichler, 'Blüthendiagramme,' Theil I, p. 65, 1875.

a terminal ovule, preceded by two or three pairs of bracts, to be homologous with a single ovule of *Cephalotaxus*, this latter being terminal to an axis whose lateral appendages have become completely abortive. He regarded the ovules of *Ginkgo* in the same light, each representing in itself a flower of which the 'collar' at the base is probably equivalent to a pair of bracts. It is probable, however, that he did not always retain these views, though I am unaware that he ever made any emendations to them.

Strasburger¹ holds the same view as Eichler, regarding the two axillary ovules of *Cephalotaxus* seated on their short axis as constituting an inflorescence, each ovule representing the remnant of a secondary ovuliferous shoot like that of *Taxus*, the primary shoot between the two rudimentary axes being entirely suppressed. For *Ginkgo* and *Phyllocladus* he likewise affords us the same explanation, but the long-stalked 'inflorescence' of the former, especially when it bears two pairs of ovules, decussately arranged, is also comparable to the entire cone of *Cephalotaxus*.

Van Tieghem's² view is very different indeed from that of either of the above authors; he interprets the axillary 'inflorescence' of Strasburger as the only *leaf* of a suppressed shoot, whose lamina has become reduced to the two ovules, in the same way as he regards the ovule of *Taxus* as the only leaf of an abortive tertiary axis. For *Ginkgo* he has precisely the same definition as for *Cephalotaxus*.

For myself, however, the views as to the morphology of the female flowers in this group which have been put forward by Čelakovský³ are the only tenable and true ones.

In their light we see that the primary shoot of *Taxus* and *Torreya* (and I have already in a previous memoir cited these various clearly-defined relationships⁴) is the homologue of the

¹ Strasburger, Angiospermen und Gymnospermen, 1879, p. 71.

² Van Tieghem, Ann. d. Sci. Nat., Bot., 5^e sér., vol. x, 1869, p. 281.

³ Čelakovský, 'Die Gymnospermen,' &c. Abhandl. d. kgl. böhm. Ges. d. Wiss., vol. iv, 1890.

⁴ Worsdell, 'The Structure of the Female Flower in Coniferae; an Historical Study.' Ann. Bot., vol. xiv, 1900, pp. 74, 75.

entire plant of *Cycads* and the brachyblast of *Ginkgo*; the secondary shoots are the homologues of the Cycadean cone and the ovuliferous axis of *Ginkgo*. In *Taxus*, *Torreya*, and *Phyllocladus* the ovule, representing in itself the entire sporophyll or carpel, has become, owing to the peculiar conditions of space-adjustment on the shoot, perfectly terminal in position on the secondary floral axis; in *Ginkgo* the two ovules, as shown so remarkably well by the abnormalities described and figured in Fujii's¹ memoir, and in that of Seward and Gowan², are really, as in *Taxus*, &c., reduced sporophylls, placed laterally on an axis whose terminal bud occasionally is formed; the sterile bracts of *Taxus*, &c. are entirely absent.

Now, if we carefully and closely regard the excessively short axillary axes, each with its two transversely-placed ovules, in *Cephalotaxus*, we notice how similar each such tiny structure is to the much-elongated ovuliferous axis of *Ginkgo*, a kind of miniature copy of the latter, the only difference between the two really consisting in the extent of development of the various parts constituting each. In fact, we may safely regard the axillary structure in *Cephalotaxus* as in itself a 'flower,' i. e. an axis bearing sporophylls, which latter have become, as in *Ginkgo*, *Taxus*, &c., reduced to ovules pure and simple. Hence the female flowers, like those on the male side, are arranged in an inflorescence, and pertain to precisely the same grade of branching, the only difference between the two inflorescences being that in the male structure the bracts are arranged spirally, in the female in decussate pairs. From all which we may gather that the 'primary shoot' of *Taxus* and *Torreya*, as also the short, fleshy ovuliferous axis of *Phyllocladus*, is the homologue of the inflorescence of *Cephalotaxus*. On this shoot there are: in *Taxus* a single fertile bract subtending a flower, and several sterile leaves; in *Torreya* a pair of fertile bracts and a single sterile one, thus

¹ Fujii, K., 'On the different Views hitherto proposed regarding the Morphology of the Flowers of *Ginkgo biloba* L.' Bot. Mag. Tokyo, vol. x, p. 7.

² Seward and Gowan, 'The Maidenhair Tree (*Ginkgo biloba* L.)' Ann. Bot., vol. xiv, 1900.

two flowers; in *Phyllocladus* several alternating fertile bracts, thus several flowers; in *Cephalotaxus* several pairs of fertile bracts. As regards each individual flower: in *Taxus* are three pairs of sterile leaves and a single sporophyll reduced to an ovule in a terminal position; in *Torreya* are two pairs of sterile leaves and a terminal ovule; in *Phyllocladus* a single terminal ovule, the entire floral axis being abortive; in *Cephalotaxus*, as in *Ginkgo*, are no sterile leaves and two *lateral* sporophylls reduced to ovules.

Here then we have the homologies and relationships between the different members of the group sufficiently clearly stated. I am of opinion that they express the true state of things much more nearly than do the views put forward by the authors cited above. I will now proceed to give some account of my own immediate observations on the female flowers of *Cephalotaxus*.

ORIGINAL OBSERVATIONS.

The tree from which the proliferating flowers were obtained stands in Windsor Park not far from Virginia Water. When quite young the tree was completely covered with a wire netting, doubtless with the object of preserving it from rabbits. This protection was, however, never removed, with the consequence that the tree during its subsequent growth became strangled and dwarfed in size, and lost its normal shape. There is a fine male tree standing near; but, although it produces flowers, pollen, I believe, is never shed; hence the flowers of the female tree could never be pollinated. I mention these two facts because it is possible that one or both of them may account for the proliferations of the female flowers.

I undertook the examination of these abnormal flowers in the hope that by means of them some direct light might be thrown on the morphology of the female flowers generally, for I am one of those who believe that abnormalities, if treated properly, may be of great value in this direction.

At the outset I may state that proliferation both of the main axis of the inflorescence or of the axis of the individual flower may take place. According to the period, early or late, at which the phenomenon occurs, will the resultant structure vary. In the case of early, congenital proliferation of the inflorescence, the latter is entirely replaced by an ordinary but very short lateral vegetative shoot bearing spirally-arranged leaves, in which no trace of bracts or ovules are visible (Fig. 3), and which itself may produce inflorescences in its turn at the base. It may frequently attain an inch in length. Such a short vegetative shoot is much more nearly comparable to a brachyblast of *Ginkgo*, and serves to show that the homology suggested above as existing between the latter and the female inflorescence of *Cephalotaxus* may not be at all far-fetched. Indeed, the frequency of these proliferated inflorescences (I have heard of their occurrence elsewhere than in Windsor Park) strengthens the likelihood of their being the homologues of the brachyblasts of *Ginkgo*. Figs. 4-8 illustrate a few of these shoots alongside normal female inflorescences, in one of which latter is a leaf below the head of bracts inserted alone, as if representing the commencement of a spiral line.

If the proliferation of the primary axis has not occurred quite so early in the development of the shoot—not, in fact, until after the formation of the first pair of bracts—we obtain the case shown in Fig. 10, where, after the pair of bracts, which may be either fertile or sterile (i. e. contain no ovules in their axils), a vegetative axis bearing spirally-arranged leaves is laid down.

Again, proliferation may set in at a much later date, viz. after the formation of two or three pairs of bracts, of which the first pair may be alone fertile, and the third pair only distinguished from the spirally-arranged leaves of the apical bud by the fact of their apices not being adpressed to the bud, but projecting slightly outward (Fig. 14). The case shown in Figs. 11 and 12 is that of an inflorescence which has made but a very feeble advance towards proliferation; the first and

lowest pair of bracts and one of the second pair have been separated from the rest by a long internode, accompanied by a displacement of some of the bracts from their paired position, which possibly may represent a transitional step from the whorled arrangement of the bracts to the spiral insertion of the leaves of the vegetative shoot.

The apical region of an unproliferated primary axis of the inflorescence is usually considerably swollen, forming a conspicuous whitish object in the midst of the whole agglomeration of bracts, &c. (Fig. 20). The swollen character is evidently due in part to the rudimentary bracts which build up this apical region, and sometimes project considerably as isolated protuberances in a lateral position and immediately above an axillary flower, so that I have often been led to imagine that this amorphous, rudimentary bract of the primary axis was an enlarged median posterior foliar organ of the axillary flower, and am still not perfectly clear as to how matters stand in all cases. This feature of the primary axis is exactly homologous with the still more swollen, fleshy character of the primary axis of *Phyllocladus*, in which the apex is equally undefined, and where several fleshy, sterile bracts frequently occur intimately fused with the axis bearing them. In fact, the inflorescence of *Phyllocladus* more nearly resembles that of *Cephalotaxus* than is the case with any other genus, the only difference being that in the former plant the bracts alternate and the floral axis is reduced to a single ovule (this being the lowest point reached in reduction of all the five genera), while in the latter genus the bracts are arranged in pairs, and the floral axis reduced to two ovules.

We next come to the consideration of the individual axillary flowers, which afford several points of considerable interest. Proliferated primary and secondary axes may occur on the same shoot, but by no means always accompany each other; indications of the latter may be present when the former are quite absent. The normal condition of things is that in which, on the excessively short axillary axis, either two lateral transversely-placed ovules are alone present, or sometimes between

these, in a median position, a rudimentary foliar organ as well. I will now proceed to cite the various instances in which this normal state of things is departed from, and shall in every case speak of the whole axillary product as a 'flower.'

In comparatively few instances, the flower bore *below* the ovules and in a lateral position a pair of foliar organs¹; in one or two cases, below these again, were rudiments of a second pair (Figs. 16 and 17). Of course, where two or three whorls are thus inserted, one immediately above and *opposite* the other, we must assume that one or more, in the latter case mentioned at least two, *median* whorls are theoretically present; but, owing to the extremely confined space existing between the bract and the primary axis of the inflorescence, have become crushed out of objective existence. However, in one flower examined, I found in the median anterior position, and below the insertion of the ovules, two exceedingly small rudiments one above the other, which quite probably represent two of the missing median whorls (Figs. 18 and 19). The appearance of these two to four whorls of foliar organs below the ovules is very interesting, for it brings *Cephalotaxus* into line with *Taxus* and *Torreya*, which always *normally* possess these inferior whorls of leaves. Indeed, these two latter plants may be considered to possess, as compared with the case of *Cephalotaxus*, somewhat proliferated floral axes. Here, therefore, already is one result of the examination of these abnormal flowers: a new link is forged between the three genera, which, with others yet to follow, will help to substantiate their nearer relationships, along lines different from those usually conceived.

The ovules are themselves frequently replaced by, or metamorphized into, foliar organs of almost the same shape and size, but always somewhat lighter in colour and more pointed at the apex than the brownish ovules. Either one or both ovules may be thus metamorphosed. These two leaves in one

¹ In every case in which either this or the term 'leaf' is used in describing the following abnormalities, it denotes a very rudimentary structure, in no way resembling, either in colour, shape or size, a foliage-leaf.

inflorescence occurred on the axillary products of both bracts of the first pair, and in each case appeared as the first two leaves of the vegetative bud representing the proliferation of the floral axis, which is subtended by each bract (Fig. 13). From their position and appearance, there could be no doubt as to their representing metamorphosed ovules in all the cases in which I observed them. This is a matter of the first importance. If the two ovules are, in reality, as appears from these abnormal cases, foliar organs belonging to the short axillary axis, the theory of Eichler and Strasburger that each ovule represents an axis in itself of which the foliar organs situated below the terminal ovule are suppressed, entirely breaks down, and the view of Čelakovský receives additional support.

Sometimes the two lateral ovules are present in the form of foliar organs, while between them, in the median position, occur either one or two ovules representing the next succeeding pair (Figs. 12, 20-22). The presence of all four ovules, two in each pair, such as has been not infrequently observed in *Ginkgo*¹, never came under my notice in the flowers I examined. We here see the opposite-decussate arrangement of the bracts on the primary axis of the inflorescence reappearing in that of the ovules and foliar organs on the secondary floral axes.

In one case which I observed, the flower consisted of the first lateral pair of foliar organs and a median pair, of which the posterior was developed as an ovule, and the anterior as a foliar organ, although of excessive minuteness (Figs. 23 and 24). Another flower exhibited a pair of lateral ovules and a median pair of organs, of which, conversely to the case just cited, the anterior was developed as an ovule and the posterior as a foliar organ (Fig. 25). In another flower, besides the lateral ovules, a pair of median organs of purely foliar nature was present (Fig. 19). In another, the median

¹ Strasburger, loc. cit., Pl. IX, Fig. 11, 1879. Čelakovský, 'Die Vermehrung der Sporangien von *Ginkgo biloba* L.' Fig. 1A, Oesterr. Bot. Zeitschr., Jahrg. 1900, Nos. 7, 8, and 9.

pair of foliar organs was reduced to a single anterior one (Figs. 28 and 29); that this latter was really anterior was shown by the fact of its concave surface being directed inwards, for I could discern no sign of the apical bud of the axis by which to orientate this leaf; it is interesting that this organ had the brown-coloured appearance usually exhibited by the ovules alone.

There came under my notice but a single example in which the floral axis simulated that of the primary axis at the apex in being swollen and enlarged and in having a foliar organ (in the posterior position) more or less fused with it.

The largest number of members ever seen on a floral axis was in a flower possessing two foliar organs and three ovules; it was impossible here to settle the true position of these five members, as displacement, arising from the confined and limited area of development, had evidently taken place.

The occurrence of a median pair of foliar organs may be regarded as an incipient stage in the transition to the complete proliferation of the axis of the flower. The next step in this direction is indicated by the case shown in Fig. 26, where, above the median pair, are two additional pairs of foliar organs. Fig. 30 represents a radial section through an axillary product at an early stage in its proliferation, showing two median leaves with the minute apical bud between them. The two lateral ovules are indicated by dotted lines. Here, although fewer foliar organs are present, the proliferation is really at a more advanced stage than in the last-mentioned case, for the axis is considerably developed and a vegetative bud already formed at the apex. Complete proliferations are shown in Figs. 13, 14, 34.

Commonly, the axillary bud causes a wide separation of the two lateral ovules as it emerges from between them, so that each projects considerably beyond the margin of the bract on either side. It also not infrequently induces the inclination of the primary axis from the vertical plane, so that the inflorescence becomes quite curved.

In one instance in which an axillary bud was present, there

were found four members belonging to the axis below the vegetative bud; these were—a lateral ovule and a lateral foliar organ (representing the normal ovule of the opposite side), a median posterior ovule, and a rudimentary foliar organ below the lateral ovule (Fig. 14). With regard to these leaves, which sometimes appear below the first normal pair of members, I may say that their frequent occurrence, either as developed or rudimentary organs, is sufficient to indicate the actual presence of an axis *below* the ovules, upon which the latter are borne as foliar members, such as is found in *Taxus* and *Ginkgo*. The occurrence (1) of these lowest foliar organs on the single axis, (2) of ovules metamorphosed into leaves¹, and (3) of the single proliferated axillary axis, are each and all of them sufficient to refute the theory of Eichler and Strasburger, much more that of Van Tieghem, and to establish the plausibility of the theory put forward by Čelakovský.

I may here conveniently submit a comparative table of the characters of the female flower of all the members of the group, which is compiled according to the views on the subject which I hold to be the true ones (p. 650). I have added thereto, at the end, the characters of the ovular integuments.

SUMMARY:

The contents of the foregoing pages may be thus summarized:—

1. *Cephalotaxus*, both in its male and female flowers, exhibits close similarities and relationships to *Ginkgo*, *Taxus*, *Torreya* and *Phyllocladus*. It is only by a careful comparative study of both normal and abnormal structures in these five genera, and of Cycads, that a true comprehension of the morphology of their flowers can be arrived at.

2. Proliferation of the primary axis of the inflorescence of

¹ The ovule being, as I hold, homologous with a leaflet or segment of a carpel or sporophyll, the structure here described as replacing the ovule, and almost identical with it in size and shape, will probably represent a sporophyll *reduced to one of its segments or leaflets* rather than an entire *foliar* structure.

Female Flowers.

<i>Torreya.</i>	<i>Taxus.</i>	<i>Ginkgo.</i>	<i>Cephalotaxus.</i>	<i>Phyllocladus.</i>
<p>'Flowers' (two or abnormally three) grouped in an inflorescence in axils of scale-leaves.</p> <p>Floral axis normally with two whorls of sterile leaves below ovules.</p> <p>'Flower' bears a single terminal ovule.</p> <p>Sporophylls reduced to ovules.</p> <p>Primary axis not vegetative.</p> <p>Floral axis not proliferated.</p> <p>Outer integument fleshy, inner woody; each distinct. (Dichlamydeous condition.)</p>	<p>'Flowers' single in axil of scale-leaf.</p> <p>Floral axis normally with three whorls of sterile leaves below ovules.</p> <p>'Flower' bears a single terminal ovule.</p> <p>Sporophylls reduced to ovules.</p> <p>Primary axis not vegetative.</p> <p>Floral axis not proliferated.</p> <p>Outer integument fleshy, inner woody; each distinct. (Dichlamydeous condition.)</p>	<p>'Flowers' single in axil of scale- or foliage-leaf.</p> <p>Floral axis with no sterile leaves below ovules.</p> <p>'Flower' normally bears one transverse pair of ovules; abnormally, a complete or incomplete median pair of ovules, or a larger number.</p> <p>Sporophylls normally reduced to ovules; abnormally developed and bearing each a terminal ovule.</p> <p>Primary axis (brachyblast) normally vegetative, bearing foliage-leaves and a terminal bud.</p> <p>Floral axis abnormally proliferated as vegetative bud.</p> <p>Outer integument fleshy, inner woody; congenitally fused together. (Holochlamydeous condition.)</p>	<p>'Flowers' (2-8) grouped in an inflorescence in axils of scale-leaves.</p> <p>Floral axis normally with no sterile leaves below whorls of sterile leaves.</p> <p>'Flower' normally bears one transverse pair of ovules; abnormally, a complete or incomplete median pair of ovules.</p> <p>Sporophylls normally reduced to ovules; abnormally, sporophylls or parts of such, replacing ovules.</p> <p>Primary axis abnormally vegetative, bearing foliage-leaves and a terminal bud.</p> <p>Floral axis abnormally proliferated as vegetative bud. Sometimes, normally, sterile leaf or two present in median position.</p> <p>Outer integument fleshy, inner woody; congenitally fused together. (Holochlamydeous condition.)</p>	<p>'Flowers' (2-8) grouped in an inflorescence in axils of scale-leaves.</p> <p>Floral axis with no sterile leaves below ovules.</p> <p>Entire 'flower' reduced to the single terminal ovule.</p> <p>Sporophyll reduced to an ovule.</p> <p>Primary axis not vegetative.</p> <p>Floral axis not proliferated.</p> <p>Outer integument fleshy, inner woody; each distinct. (Dichlamydeous condition.)</p>

the female flower may occur, in greater or less degree, according to the period at which it sets in.

3. Proliferation of one or more secondary or 'floral' axes may occur. The various tendencies to proliferate are shown in the metamorphosis of the ovules into foliar organs, the occurrence of foliar organs, rudimentary in character, both above and below the insertion of the ovules.

4. Each female 'flower' is shown by the proliferations to be an axillary axis bearing foliar organs arranged on the opposite-decussate plan, being in this respect a reproduction of the primary axis.

In conclusion, I may say, that it is to the acute powers of observation of Miss Benson and, I believe, of one of her pupils, Miss Sanday, who first detected the abnormal flowers, that I am indebted for any results which may have accrued from their examination.

EXPLANATION OF FIGURES IN PLATE XXXV.

Illustrating Mr. Worsdell's paper on *Cephalotaxus*.

- Fig. 1. Male inflorescence; from a photograph. Nat. size.
Fig. 2. Male sporophyll or stamen.
Fig. 3. Shoot with proliferated female inflorescence *in situ*. Nat. size.
Fig. 4. Normal female inflorescence. Nat. size.
Fig. 5. Sub-normal inflorescence with a bract or leaf halfway up the stalk. Nat. size.
Figs. 6-8. Congenitally proliferated female inflorescences. Nat. size.
Fig. 9. Diagram of a normal female inflorescence.
Fig. 10. Proliferated primary axis of inflorescence; one pair of bracts present. $\times 7$.
Fig. 10a. Diagram of the same.
Fig. 11. An inflorescence, showing a slight indication of proliferation. $\times 3\frac{1}{2}$.
Fig. 12. Diagram of the same.
Fig. 13. An inflorescence, showing proliferation both of the primary and two secondary or floral axes. $\times 7$.
Fig. 14. An inflorescence with proliferation of primary and of one secondary axis; in two of the flowers a foliar organ occurs below a lateral ovule; in the flower on the right a median posterior ovule is present. $\times 7$.
Fig. 15. Diagram of the same.

Fig. 16. Tangential section of a flower, showing a foliar organ below each of the lateral ovules, and rudiments of others still lower on the axis. $\times 20$.

Fig. 17. Diagram of the same.

Fig. 18. Radial section of a flower, showing two median rudimentary foliar organs; the larger organ δ is probably a median bract of the inflorescence. $\times 30$.

Fig. 19. Diagram of the same.

Fig. 20. An inflorescence with the insertion of three pairs of bracts indicated; in the median flower one of the lateral ovules is replaced by a foliar organ, and a median ovule is also present; the bract removed. $\times 7$.

Fig. 21. Flower with three ovules; bract removed. $\times 7$.

Fig. 22. Flower with both lateral ovules replaced by foliar organs, and a median ovule present; bract removed. $\times 7$.

Fig. 23. Radial section of a flower, showing position of a median ovule and foliar organ. $\times 20$.

Fig. 24. Diagram of a flower with the two lateral ovules replaced by foliar organs, and with a median ovule and foliar organ.

Fig. 25. Diagram of a flower with median ovule and foliar organ.

Fig. 26. Diagram of a flower with one of the lateral ovules replaced by a foliar organ, and with two median pairs and one transverse pair of foliar organs—the most primitive stage in the proliferation of the flower.

Fig. 27. Diagram of a flower in which lateral ovules are replaced by foliar organs, and with a median pair of foliar organs and ovules.

Fig. 28. Flower in axil of bract with one of the lateral ovules removed (indicated by dotted line) to show a median foliar organ. $\times 10$.

Fig. 29. Diagram of the same.

Fig. 30. Radial section of a flower, showing an early stage of proliferation; the lateral ovules indicated by dotted lines. $\times 30$.

Fig. 31. Diagram of the same.

Fig. 32. Radial section of a flower, showing two median foliar organs. $\times 20$.

Fig. 33. Ditto, showing median posterior foliar organ and axis of flower. $\times 30$.

Fig. 34. Two proliferating axes, one of which is a floral axis, the other of uncertain position.

The following are the abbreviations used :—

ap. bd = Proliferating apex of the primary axis.

ax. bd = Proliferating apex of the floral axis.

lr = Rudiment of foliar organ.

l = Foliar organ situated below insertion of lateral ovules.

*l*¹ = Foliar organ which replaces a lateral ovule.

*l*² = Median foliar organ.

*ov*¹ = Lateral ovule.

*ov*² = Median ovule.

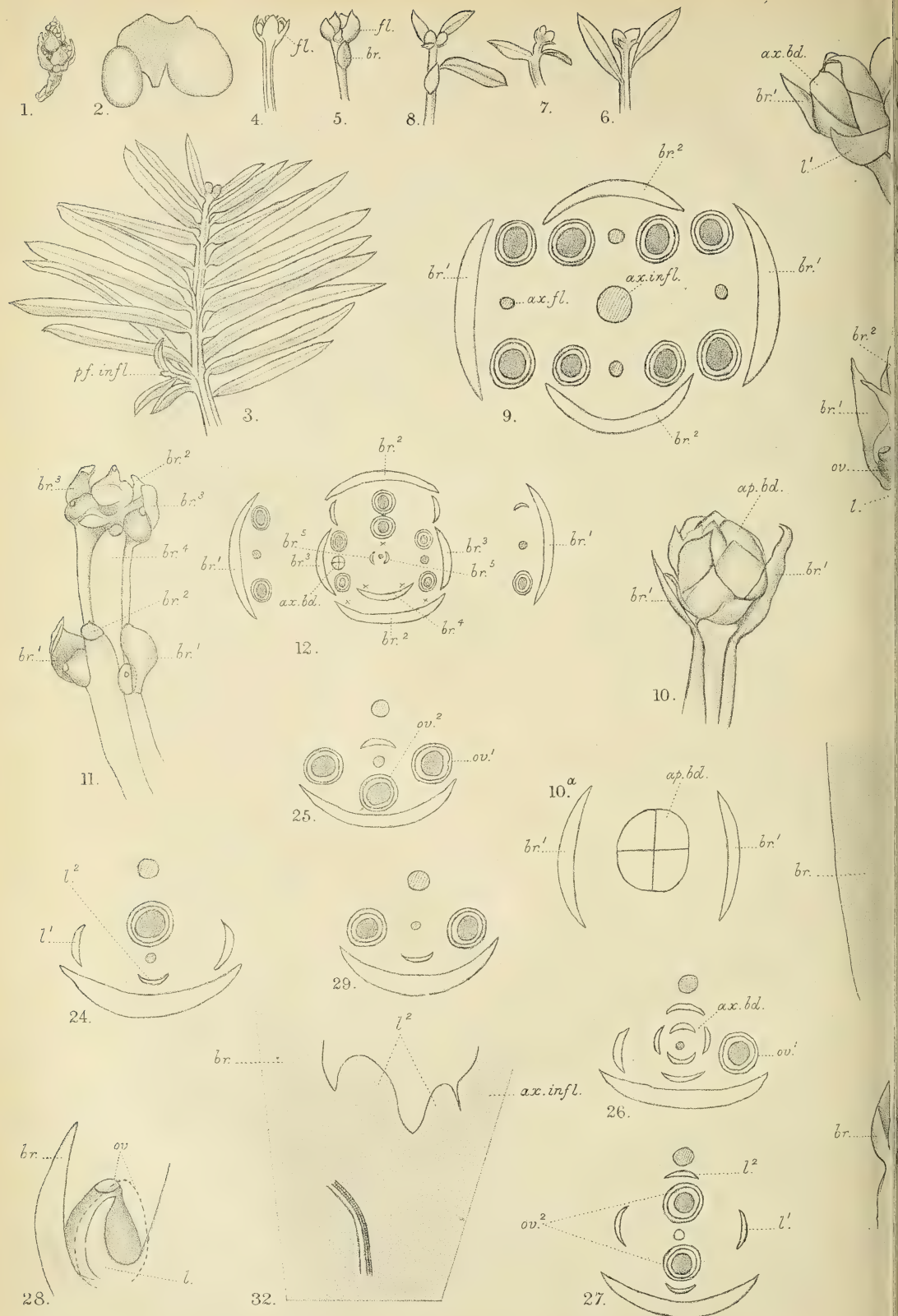
*br*¹, *br*², *br*³, *br*⁴, *br*⁵ = Bract of 1st, 2nd, 3rd, 4th, and 5th pair respectively.

ax. infl. = Primary axis.

ax. fl. = Floral axis.

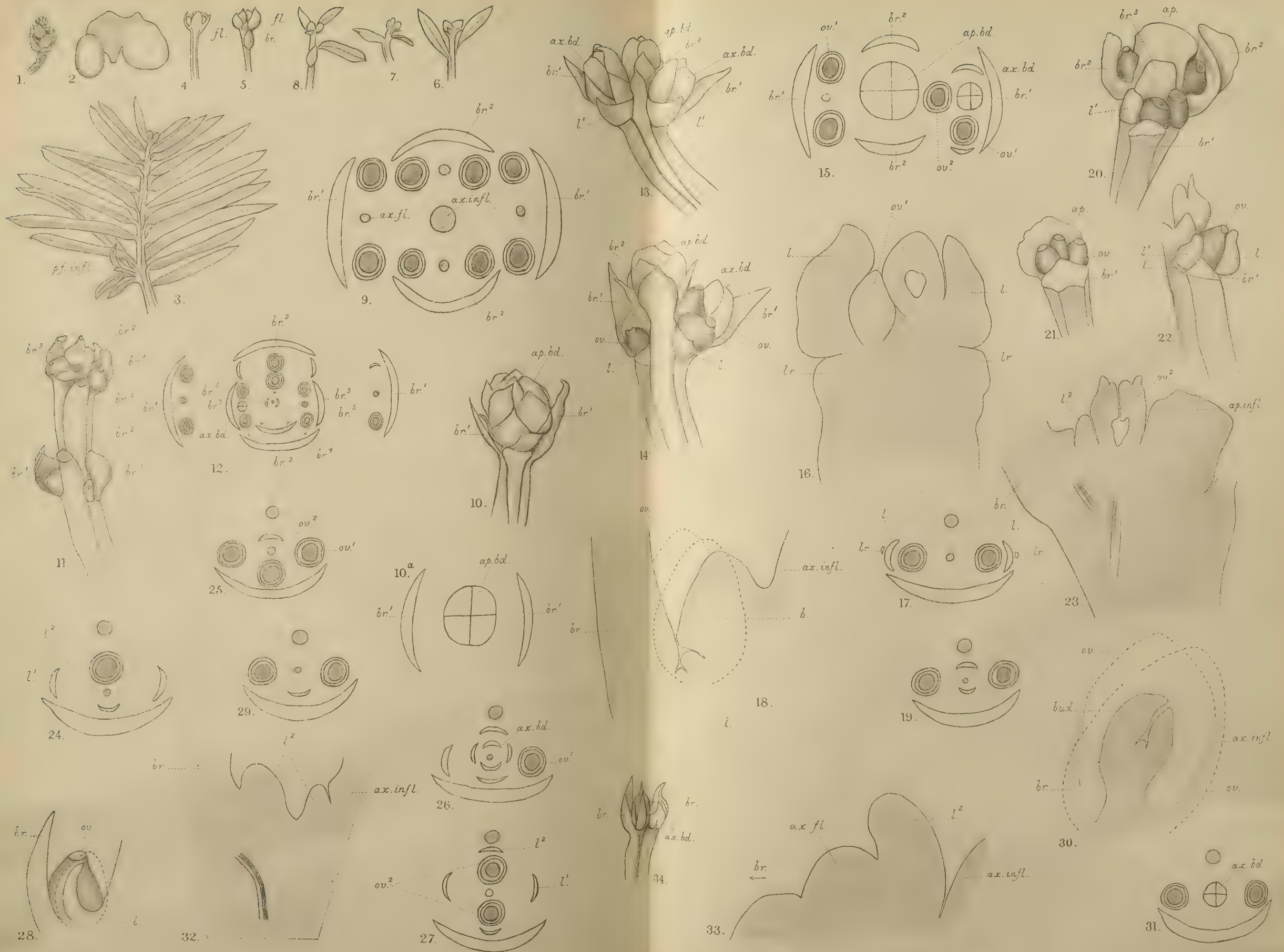
pf. infl. = Proliferated inflorescence.

x = Position of some missing structure, either ovule or bract.



W.C.W. del.





The Fertilization of *Pythium de Baryanum*.

BY

KIICHI MIYAKE, M.A.

—♦—

With Plate XXXVI.

—♦—

PRINGSHEIM ('58) was the first who observed the phenomena of fertilization in *Pythium*. He found that in *Pythium monospermum*, the antheridium sends out a process into the oogonium, while the contents of the latter separate from the wall and accumulate into a spherical mass, the oosphere, which he calls the 'Befruchtungskugel.' After the fertilization-tube reaches the oosphere, it discharges its contents through the opening at the apex. He believed that very minute spermatozoid-like bodies in active motion were found in the antheridium. Cornu (72') observed the passage of the antheridial contents into the oogonium in *Pythium gracile* and *P. Cystosiphon*, but he denied the presence of spermatozoid-like bodies in the antheridium. After that De Bary ('81) made more careful studies and found that, in several species of *Pythium*, soon after the formation of the fertilization-tube the protoplasm becomes differentiated in the antheridium; the larger and denser granular portion moves into the centre of the cavity, forming there an irregular and somewhat indistinct strand, the gonoplasm, while a thin layer, the periplasm, remains next the wall of the cell. Then the gonoplasm passes slowly through the fertilization-tube which has

[Annals of Botany, Vol. XV. No. LX. December, 1901.]

opened in the meanwhile into the oosphere. His results were confirmed by Ward ('83 b), Atkinson ('95), and others.

Fisch ('85) studied for the first time the nuclear phenomena in the fertilization of *Pythium*. He used various solutions of Haematoxylin for the stain, and found that in the young oogonium there are usually from ten to twenty nuclei. At the time of the oosphere-formation, he thought, they fused together into a single large nucleus. In the antheridium he always found a single nucleus, but thinks that this may be due to the fusion of several. The nucleus of the antheridium passes into the oosphere with the gonoplasm and fuses with the egg-nucleus.

Later, Dangeard ('90, '92) made some observations on *Pythium monospermum* and *P. proliferum*, in which he showed that the antheridia and oogonia are multinucleate, but he was unable to follow the fate of the nuclei to the time of fertilization. He also pointed out that a central oleaginous globule appears in the oogonium at a certain stage, and that this was probably mistaken by Fisch for a nucleus, and led him to the conclusion that all the nuclei of the oogonium fused together to form a single sexual nucleus.

These are the only papers concerned with the nuclear phenomena of fertilization in *Pythium*, so far as I know. In the light of recent investigations on the fertilization of Peronosporae, some of Fisch's results seem to be very doubtful, and Dangeard's studies do not seem to give much light on this point.

The present investigation was undertaken in the Botanical Laboratory of Cornell University, under the direction of Professor George F. Atkinson, to whom I wish to express my hearty thanks for his kind advice and suggestions.

MATERIAL AND METHODS.

The species I have studied was *Pythium de Baryanum*, a fungus which is responsible for a large part of the 'damping off' of young seedlings. It is very widely distributed, being common in the soil of gardens and fields. I collected earth from a rather low part of a garden, placed it in pots and boxes

in the greenhouse, and planted in it some cucumber (*Cucumis sativus*, L.) seeds. Within two or three days after germination of the seeds, some of the seedlings began to droop, the tissues softening and yielding near the surface of the soil. The prostrate plants were found to be more or less shrunk and collapsed at this point, so that on pulling, the plant broke easily. The Fungus seems to attack the root and the lower part of the stem first, then to proceed upwards, so that in the course of a few days the entire plant wilts and finally decays. If a portion of the collapsed part of the stem is teased apart on the slide and examined under the microscope we can find the abundant colourless branched mycelium of the Fungus extending in the direction of the axis of the stem, and sometimes young oogonia can be seen here and there, in the part which is much decayed.

I then took the hypocotyl of the diseased seedlings, usually choosing one which was not very much collapsed, and placed it in a Petrie dish on moistened filter-paper. After one or two days sexual organs are abundantly formed, older stages being usually found in the lower part of the hypocotyl which was first attacked by the Fungus. Before fixing, small portions of the different parts of the hypocotyl were examined under the microscope in order to see that a number of sexual organs were present in different stages. The suitable portions were cut into pieces 5 to 7 mm. in length, and fixed.

The fixing fluids used were chrom-osmium acetic acid; chrom-acetic acid; corrosive sublimate in aqueous solution both hot and cold; acetic-sublimate solution in alcohol or water; absolute alcohol; Carnoy's fluid, and Merckel's fluid. The first two gave the best results, and the second one was more extensively used.

The material was usually left in the fixing solution from fifteen to twenty-four hours, then washed in several changes of water for ten or more hours. It was then gradually transferred through the usual grades to absolute alcohol. After dehydrating it was brought very gradually into xylol or chloroform, and transferred with equal care into paraffin, with

a melting-point of 54° , in which it was finally imbedded. Sections, from 2 to 5μ in thickness, were cut with a Minot-Zimmermann revolving microtome, and fastened to the slide with albumen fixatives.

Among several staining combinations used, Flemming's triple stain gave the best results, and it was very extensively used throughout the research. Heidenhain's iron-alum Haematoxylin was sometimes used, and it gave some good results. Hartog's ('95) nigrosin-carmin stain, as used by Wager ('96) and Berlese ('97), was tried on material fixed in sublimate solutions, but the results were not very satisfactory.

DEVELOPMENT OF THE SEXUAL ORGANS.

Nuclei are found irregularly distributed in the protoplasm in all parts of the mycelium. The shape of the nucleus is spherical or more or less oval (Plate XXXVI, Fig. 1). But in some parts of the mycelium the nuclei become considerably elongated and deformed. They contain the granules of chromatin, and no nucleoli seem to be present, so far as I am able to determine by the staining reactions. No haustoria are found in any part of the mycelium.

The oogonia are formed as terminal or intercalary swellings of the hyphae (Figs. 2, 3). Large quantities of protoplasm and numerous nuclei pass into them from the hyphae, and when they have reached their full size they are cut off from the hyphae by a wall, or, if their position is intercalary, by two transverse walls (Figs. 4, 5, 6).

About the same time that the oogonia are cut off one or more antheridia begin to develop close to each oogonium. The antheridia are of two kinds, stalk-antheridia and branch-antheridia. The stalk-antheridium, the simpler of the two, is formed from the part of the hypha next to the oogonium, by cutting off an elongated cell, one end of which is thus in contact with the wall of the oogonium, and its contents are only separated from those of the oogonium by the wall of the latter, as in *Monoblepharis*. The terminal oogonium is therefore placed upon it as on a stalk-cell, which may either

remain straight or have a characteristic curvature (Figs. 7, 13). In the intercalary oogonium, two stalk-antheridia may develop, one on either side of the oogonium.

The branch-antheridium is the one more commonly found. It is a terminal cell of a special branch of the hyphae, arising usually as a lateral branch of the hypha supporting the oogonium, and very often quite near the latter (Figs. 4, 8, 11). But sometimes, though rarely, it develops from a separate hypha near the oogonium. The antheridial branch grows towards the oogonium, and its rounded end finally comes in contact with the wall of the latter (Figs. 7-11). A septum is now formed in the branch, cutting off an elongated curved cell, the antheridium. More than one antheridium may be found in connexion with a single oogonium, frequently two antheridia (Fig. 10) and sometimes three.

A young oogonium, which is about 20 to 25 μ in diameter, contains approximately from ten to fifteen nuclei. The nuclei are more or less spherical, being larger than those of the hyphae. The chromatin granules are more prominent, and no nucleoli are found. It is very difficult to count the number of nuclei in the antheridium, owing to its small size and irregular shape. The number seems to vary from two to six.

The division of the nuclei takes place almost simultaneously in the oogonium and antheridium (Figs. 13-17). In the oogonium, the nuclei, before division, arrange themselves near the periphery. The division of the nucleus is karyokinetic and is very similar to that which takes place in *Cystopus* and *Peronospora* as described by Wager ('96, '00), Stevens ('99), Davis ('00) and others. The several karyokinetic figures are shown in Fig. 25.

The chromatin granules first of all appear to be transformed into a number of chromosomes in the shape of very short rods or oval bodies. By the formation of the spindle they arrange themselves in the equatorial plate (Fig. 25 a). The number of chromosomes is very difficult to count, but in several cases I was able to count as many as eight. The nuclear membrane seems to remain intact, at least in

the early stages of the division, as in *Cystopus* observed by Wager, Stevens and Davis. No definite centrosomes are seen, although occasionally granules at the poles of the spindle were observed which might have been taken for such, as is shown in Fig. 25*a*. It is more difficult to study the division in the antheridium. The process does not seem to differ from that which is found in the oogonium. Fig. 25*c* shows one of the karyokinetic figures found in the antheridium.

This division of the nuclei in the oogonia and antheridia previous to fertilization is exactly similar to that which occurs in *Cystopus* and *Peronospora*, and according to Trow ('95, '99) in *Saprolegnia* and *Achlya* also. A similar phenomenon has been described by Eidam ('86), Chmielewsky ('88) and Fairchild ('97) in *Basidiobolus*. The significance of this division is not yet very clear. Hartog ('91) suggests that 'we can regard the nuclear divisions in oogonium and antheridium as phylogenetic reminiscences of the formation of gametes by cell-division.' Wager ('96) in the brief discussion of this subject in his paper on *Cystopus candidus* says that 'the readiest explanation of the phenomenon would of course be that it is a reducing division, but this does not appear probable in the light of recent investigations on the reduction of the chromosomes in plants and animals, and also from the fact that, so far as I was able to observe in *Cystopus*, the number of chromosomes in the dividing nuclei in the oogonium is less than the number in the dividing nuclei of the fully formed oospore.' In his recent paper on *Peronospora* Wager ('00) is inclined to regard it as a reducing division, since he states: 'but it appears to me, from the few observations which I have been able to make, that a reduction in the number of chromosomes takes place during the mitosis in the oogonium and probably in the antheridium.' Berlese ('97) believes that the reduction does not take place in the division of the sexual nuclei, but that it occurs, at least in *Cystopus Portulacae*, which he has actually observed, on the germination of the oospore. But Stevens ('99) says that in *Cystopus (Albugo) Bliti* 'there is some evidence that makes it appear that there

is a reduction in the number of chromosomes during the first mitosis.' It is evident that further investigations on the division of sexual as well as vegetative nuclei are necessary, before any definite explanation can be given as to the significance of the nuclear division in the gametangia.

FORMATION OF THE OOSPHERE AND FERTILIZATION.

While the nuclei arrange themselves in the peripheral part of the oogonium, the central part of the protoplasm becomes more or less vacuolate. After the division of the nuclei, the entire protoplasmic contents differentiate into ooplasm and periplasm (Fig. 17). In *Cystopus* and *Peronospora* this differentiation of the protoplasm into two parts takes place a little earlier than in *Pythium*; it occurs just about the time of nuclear division, or a little before. I have failed to observe any differentiated mass of protoplasm in the centre of the ooplasm, as found in all the species of *Peronosporae* so far studied, and named by Stevens the coenocentrum.

A single nucleus enters into the ooplasm and remains usually at the centre, while all the other nuclei are left in the periplasm and finally degenerate (Fig. 18). Now the oosphere with its nucleus is formed, but no wall seems yet to be formed around it.

It is interesting to note here that in *Cystopus Bliti*, according to Stevens ('99), the oosphere is multinucleate, and the nuclei divide once more before they fuse with the nuclei from the antheridium; the same state of things was found, by the recent investigations of Stevens ('01), in *Cystopus Portulacae*, whose oosphere was described by Berlese ('97) as uninucleate. Stevens ('01) also found that in *Cystopus Tragopogonis*, the oosphere is multinucleate and the nuclei divide again previous to fertilization, but only one of them becomes the functional nucleus of the oosphere, while all the other nuclei degenerate. This species is considered to be a connecting link between the above-mentioned two species of *Cystopus* and *C. candidus* whose oosphere is uninucleate from the beginning. Stevens' recent researches on *Cystopus candidus* differ from those of

Wager ('96) and Davis ('00) in one essential point. Stevens ('01) found that in *Cystopus candidus* the nucleus of the oosphere was derived from one of the nuclei which remained in the ooplasm from the time of its differentiation, instead of coming to the non-nucleated centre of the ooplasm from the periphery. Thus he states that at no time of its development is the central region of the oosphere entirely free from a nucleus, and in this respect *Cystopus candidus* differs from the other species of the genus.

In the antheridium, after division, all the nuclei degenerate except one which becomes enlarged (Fig. 18). While the oosphere is differentiating, the antheridium puts out a fertilization-tube from the point of attachment into the oogonium, piercing through the wall of the latter and pushing its way through the periplasm into the ooplasm (Figs. 19-22). The fertilization-tube is a thin-walled cylindrical tube, often somewhat narrowed at the end, instead of being enlarged as in *Peronospora* and *Cystopus*. The tube does not seem to reach deep into the centre of the oosphere, as in many species of *Peronosporae*, but it appears to open near the periphery of the oosphere. I am not, however, very certain on this point, as in a few preparations something like the fertilization-tube was seen to reach the centre of the oosphere from the periplasm.

The greater part of the antheridial contents with the male nucleus passes gradually into the oosphere through the fertilization-tube (Fig. 19). The nucleus of the antheridium, after entering the oosphere, comes in contact with the female nucleus in the centre, and then they fuse together to form the nucleus of the oospore (Figs. 20-22).

It would be interesting to study the behaviour of the nuclei during fertilization in the oogonium with more than one antheridium. De Bary ('84) mentions that, if there is more than one antheridium, they all usually, but not always, empty their gonoplasm one after another into the oosphere. My present investigation has so far failed to make this point clear.

MATURATION OF THE OOSPORE.

Soon after the discharge of the antheridial contents into the oosphere, a thin membrane is formed around the latter (Figs. 20-22). This is the beginning of the exospore. After fertilization it gradually thickens until it reaches about the thickness of the oogonium-wall. Figs. 23 and 24 show the oospore in which the formation of the exospore is nearly completed. In this stage only a very small quantity of protoplasm remains in the periplasm. It is very likely that the greater part of the periplasm has been used up in the formation of the exospore.

Later the endospore begins to be formed inside the exospore. Fig. 25 shows an exospore in which the endospore is about half way thickened, and in Fig. 27 we can see the endospore is nearly completed. The exospore is more deeply stained than the endospore.

The ripe oospore is uninucleate, and in the nucleus several deeply-stained chromosome-like bodies are clearly visible (Figs. 26, 27). The protoplasm is vacuolate in structure, showing a regular net-work in the section, and it takes the stain more readily. The wall of the oogonium which thickens in the course of the development of the latter, but very slightly or scarcely at all after fertilization, persists as an additional protection of the oospore (Fig. 27, &c.).

From the foregoing statements we can see that, in the mode of sexual reproduction, *Pythium* has more resemblance to the Peronosporae than to the Saprolegnieae. In some species of Saprolegnieae, the oospore is formed parthenogenetically, the antheridia being very rare or never developed. In others, which have well-developed antheridia, the existence of fertilization is still in doubt. De Bary ('81) first expressed the doubt, and after careful observations came to the conclusion that the Saprolegnieae as a group are distinctly parthenogenetic. His conclusion was based on the failure to observe any passage of protoplasm from the fertilization-tube to the oosphere, or even any opening in the tube. Pringsheim ('82, '83)

opposed this view, and maintained that in certain species he actually observed the fertilization taking place by the amoeboid swimmers—'Spermamoebae'—developed in the fertilization-tubes and set free from them. His observations were objected to by Zopf ('82, '83), De Bary ('83), Ward ('83 *a*) and others.

Hartog ('89, '91, '95), Dangeard ('90, '91), Humphrey ('92), and Trow ('95, '99) studied the nuclear phenomena of this group. Hartog and Humphrey agree in the absence of fertilization, Dangeard leaves this question undecided, while Trow maintains that in certain species at least true fertilization takes place.

In the Peronosporae, on the other hand, the true process of fertilization, i. e. the fusion of sexual nuclei, takes place in all the species so far studied. The results of De Bary's ('81) classical researches, confirmed by the recent cytological investigations of Wager ('89, '96, '00), Stevens ('99, '01), Davis ('00) and others leave no room for doubt on the existence of fertilization in this group.

The question naturally arises as to which of these two groups of Fungi *Pythium* is most closely related. Pringsheim ('58), who established the genus *Pythium*, placed it among the Saprolegnieae. Cornu ('72) in his monographic studies of Saprolegnieae, included *Pythium* in that group. Later, De Bary ('81) placed this genus under the Peronosporae, and this arrangement has been accepted by later students of Fungi. More recently, Schröter ('93), in Engler and Prantl's 'Pflanzenfamilien,' classified *Pythium* as a member of Saprolegnieae, placing it under the separate family Pythiaceae. The reason for adopting the new classification is given by him as follows: 'Die Pythiaceae zeigen eine sehr nahe Verwandtschaft zu den Peronosporineae, besonders in Hinsicht auf die Befruchtung und die Oosporenbildung. Sie sind darum auch von De Bary mit den Peronosporineae vereinigt worden. Habituell stehen sie den Saprolegniineae näher, und es erscheint in einem System, welches nicht mehr einsichtig auf ein einzelnes Merkmal (die Sexualität), sondern auf die

Berücksichtigung aller Merkmale begründet ist, passender, sie im Zusammenhang mit den S. zu besprechen, als besondere Familie, welche in der Mitte zwischen beiden steht.'

As remarked by Schröter, it is incorrect to base the classification solely on the mode of sexual reproduction. But the sexual organs are usually regarded as the most important characters used in classification, since the vegetative parts of the plant-body are more liable to modification according to the conditions of environment to which the plants are subjected, while the reproductive organs undergo less change. The present studies show more definitely the close relationship of the Peronosporae and *Pythium* in the mode of sexual reproduction.

It appears therefore that its natural position would be in the order Peronosporae rather than in the Saprolegnieae, although it occupies an intermediate position between the two.

SUMMARY.

1. The mycelium of *Pythium de Baryanum* contains numerous nuclei irregularly distributed in the protoplasm.

2. The young oogonium, which is formed as either a terminal or an intercalary swelling on the hypha, contains about 10 to 15 nuclei. The nuclei are larger than those of the hyphae, and the chromatin granules are prominent in stained preparations.

3. The antheridium contains about 2 to 6 nuclei. The structure of the nuclei does not differ from that of the nuclei in the oogonium.

4. The nuclei of the oogonium and antheridium undergo division previous to fertilization. The division is karyokinetic, and similar to that which takes place in *Cystopus* and *Peronospora*. The formation of the karyokinetic spindle is intranuclear, and the nuclear membrane persists at least in the early stages of the division.

5. After the division of the nuclei which were previously arranged near the periphery of the oogonium, the whole

contents of the oogonium differentiate into the ooplasm and periplasm. Then one nucleus from the periphery enters into the ooplasm to become the functional female nucleus of the oosphere, while all the other nuclei in the periplasm degenerate there.

6. The nuclei of the antheridium after division degenerate, except one which remains to become the functional male nucleus. While the formation of the oosphere is taking place in the oogonium, the antheridium sends out a fertilization-tube. The tube reaches to the oosphere, piercing through the wall of the oogonium and periplasm.

7. The greater part of the contents of the antheridium, including the male nucleus, passes gradually into the oosphere, through the fertilization-tube. The male nucleus finally comes in contact with the female nucleus, and they fuse together to form the nucleus of the oospore.

8. After the discharge of the antheridial contents into the oosphere, the exospore begins to form around the latter. The greater part of the periplasm seems to be used in the formation of the exospore which has about the same thickness as the oogonium-wall, when completed. A much thicker endospore develops later inside the exospore. The ripe oospore is uni-nucleate, and in the stained section several chromosome-like granules are visible in the nucleus.

CORNELL UNIVERSITY,

June, 1901.

After this was written, Dr. Trow's paper, 'Observation on the Biology and Cytology of *Pythium ultimum*, n.sp.,' appeared in *Annals of Botany* (June, 1901). His results agree with mine in essential points, but differ somewhat in matters of less importance.

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EXPLANATION OF FIGURES IN PLATE XXXVI.

Illustrating Mr. Miyake's paper on the Fertilization of *Pythium*.

All figures were sketched with the aid of the camera lucida, using Bausch and Lomb's $\frac{1}{2}$ in. oil immersion objective with ocular 1 ($\times 1300$), except Fig. 28, which was drawn with Zeiss' apochromatic 1.5 mm., 1.30 apert., homog. immersion objective, compensation ocular 12 ($\times 2700$).

Fig. 1. Portions of mycelium showing several nuclei.

Fig. 2. The formation of an intercalary oogonium.

Fig. 3. The formation of a terminal oogonium.

Fig. 4. Intercalary oogonium with an antheridium attached in one side.

Figs. 5-6. Terminal oogonia.

Fig. 7. Young oogonia with a stalk-antheridium.

Figs. 8-9. Young oogonia with branch-antheridia.

Fig. 10. An oogonium with two antheridia.

Fig. 11. An oogonium with a branch-antheridium.

Fig. 12. An oogonium with the nuclei dividing near the periphery.

Fig. 13. An oogonium with a stalk-antheridium, the nuclei of both are dividing or just about to divide.

Fig. 14. An oogonium with a branch-antheridium, the nuclei of both are just dividing.

Fig. 15. An antheridium, showing a dividing nucleus. Only a part of oogonium is shown.

Fig. 16. An oogonium with a branch-antheridium, the stage a little later than Fig. 14.

Fig. 17. The contents of an oogonium as just differentiating into the ooplasm and periplasm.

Fig. 18. Later stage: the oosphere is formed, showing the degenerating nuclei in the periplasm. The antheridium with one functional nucleus and several degenerating nuclei is shown in one side.

Fig. 19. The antheridium discharging the contents: the male nucleus just entered the oosphere.

Fig. 20. The male nucleus is approaching the female nucleus. The thin wall is formed around the oosphere.

Fig. 21. Two sexual nuclei are just in contact.

Fig. 22. A stage same as Fig. 20, with a stalk-antheridium.

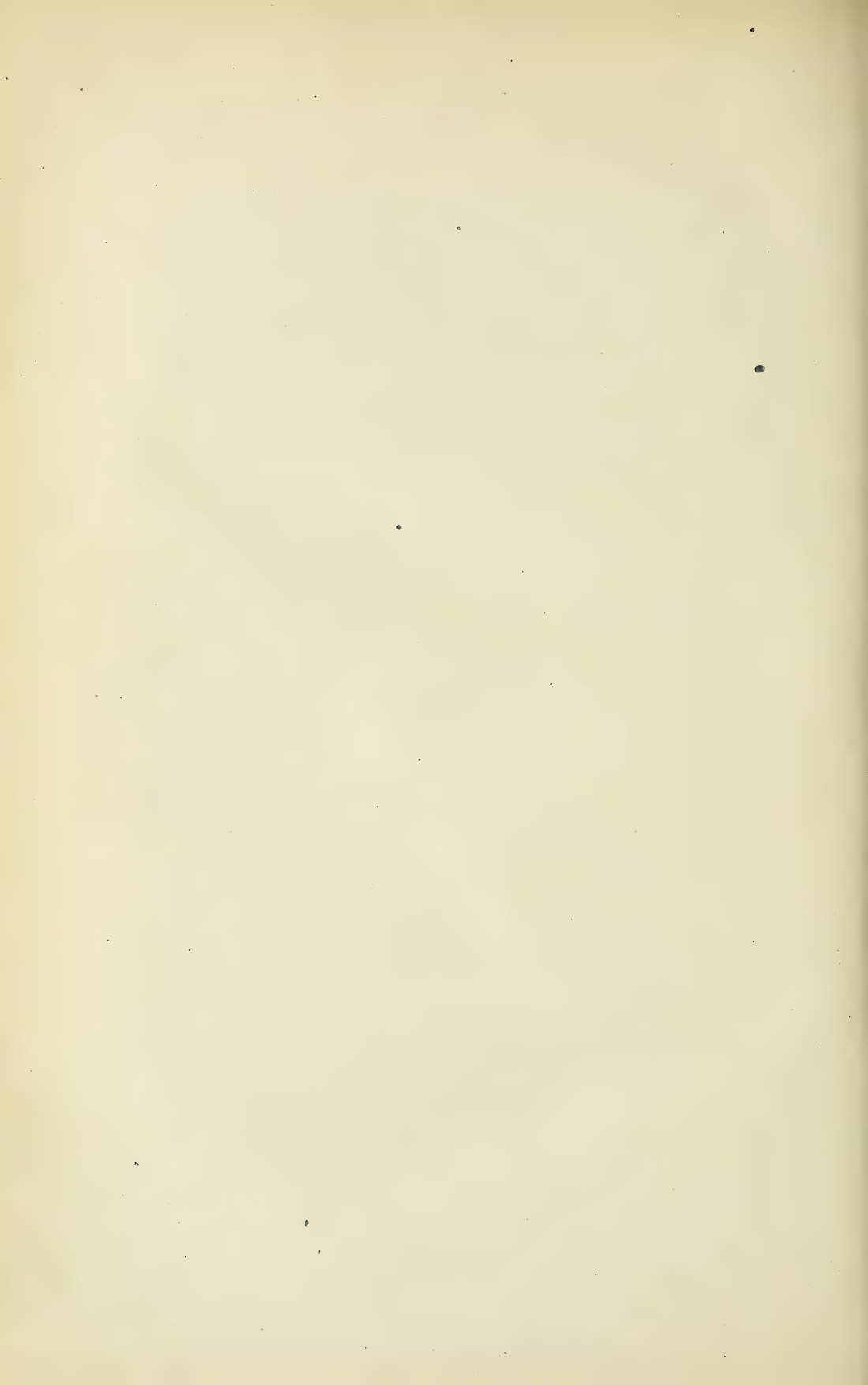
Figs. 23-24. Young oospores each with a single nucleus, and the exospore are nearly complete.

Fig. 25. An oospore with the endospore about half-way thickened.

Fig. 26. An oospore in a stage older than Fig. 25.

Fig. 27. Nearly ripe oospore with oogonial wall, emptied antheridium still attached.

Fig. 28. Several karyokinetic figures. *a, b, d, e* in oogonium, *c* in antheridium.





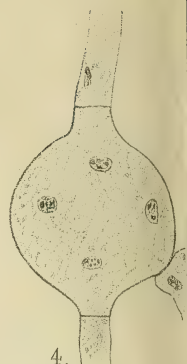
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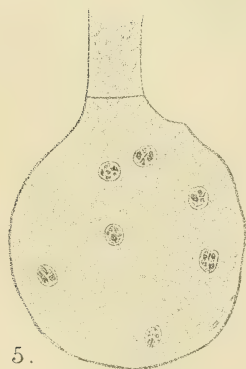
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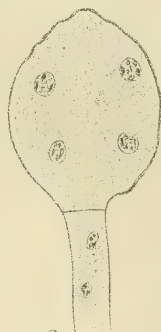
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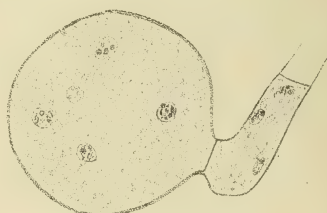
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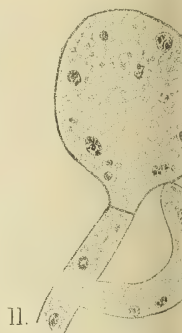
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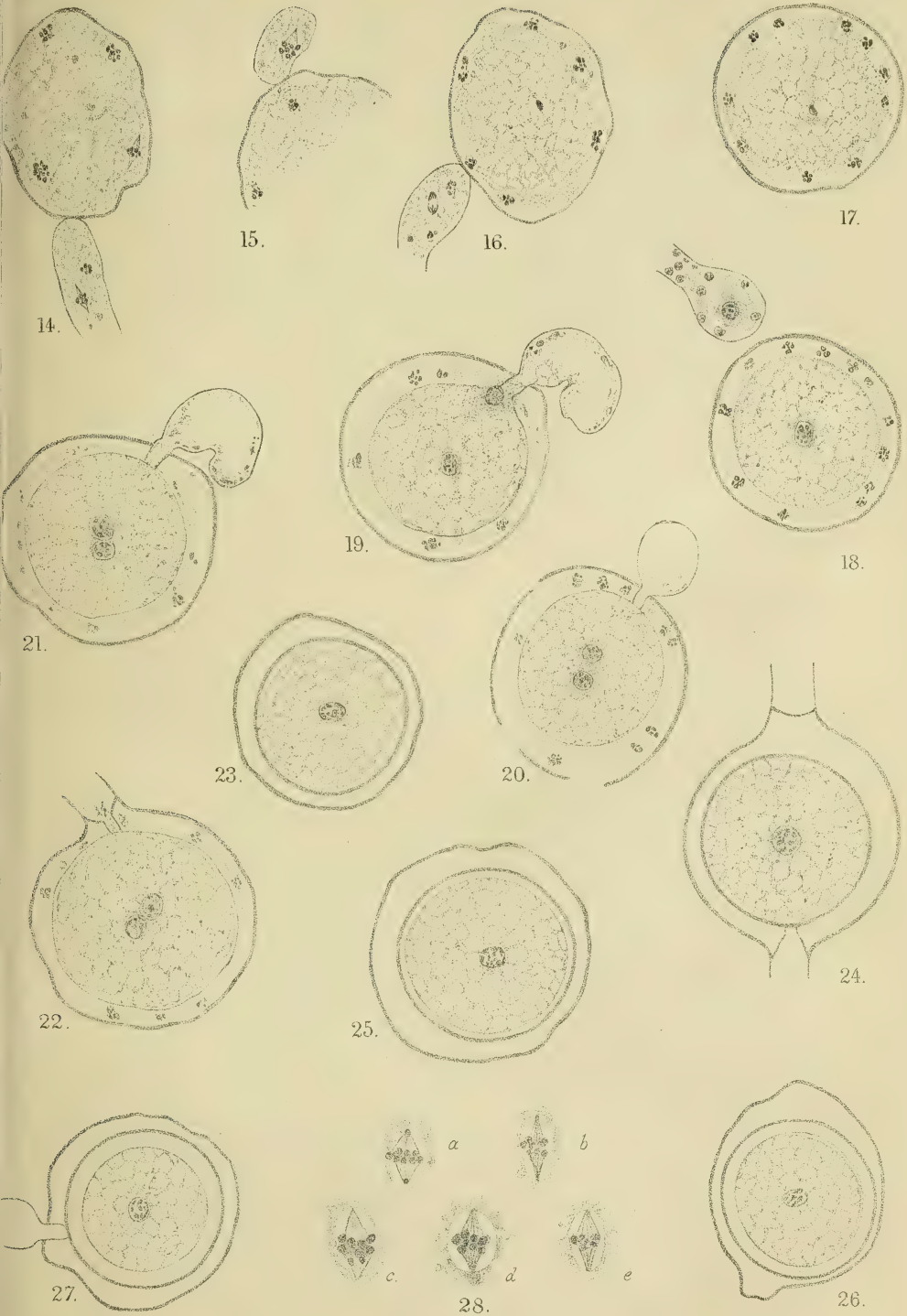
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13.





On the Effect of Nitrates on the Carbon-Assimilation of Marine Algae.

BY

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IN a former paper¹, some account was given of experiments on the effect of salts on the assimilation of carbon dioxide in *Ulva latissima*, L. The object of that work was to obtain some qualitative idea of the relative value of the principal salts of sea water in regard to the maintenance of carbon-assimilation. The salts, then made use of, were the chlorides and sulphates occurring in sea water. In the present communication will be found an account of experiments with nitrates, and other salts, on the same Alga by a similar method, and of attempts to extend the work to other marine Chlorophyceae.

NITRATES.

Molisch² and Kossowitsch³ have shown that the higher Algae obtain their nitrogen entirely in the combined form. Very little is known of the influence of nitrates on marine Algae. With fresh-water Algae, the effect of the presence of different nitrogenous compounds in the medium seems to vary

¹ Arber ('01), p. 39.

² Molisch ('95).

³ Kossowitsch ('94).

greatly with the nature of the salt, and with different Algae. Loew and Bokorny¹ found that *Spirogyra* flourished better in sodium nitrate (NaNO_3) than in potassium nitrate (KNO_3). The presence of KNO_3 induced an abnormal amount of starch-formation, but quickly resulted in the death of the Alga. Ammonium salts were found to be directly injurious to *Spirogyra*; an addition of 0.1 per cent. NH_4Cl quickly caused death. Stange² says that *Spirogyra*, *Cladophora*, and *Zygnema* will never tolerate more than 1 per cent. KNO_3 , and *Oscillaria* 1.5 per cent.; while species of *Pleurococcus* have been observed to thrive in 12 per cent. KNO_3 . In the higher plants, Stange also found that a solution of KNO_3 gave disastrous results in many cases, and Halophytes were generally quickly killed by a solution containing 0.10 gram-molecules per litre KNO_3 . Quite recently Jacobi³ has shown that potassium nitrate decreases the assimilation of submerged plants, such as *Elodea* and *Myriophyllum*.

The nitrate which occurs in sea water is magnesium nitrate, and this is undoubtedly the sole source of nitrogen to such Algae as *Ulva*. The amount is exceedingly small, about .002 per cent. During the course of this work I made a considerable number of experiments with four nitrates, viz. those of potassium, sodium, magnesium, and ammonium, each being used separately. Two series of experiments were made in each case, with the exception of ammonium nitrate. In one, a certain percentage of the nitrate alone was dissolved in *distilled* water, in the other, in *sea* water.

(A) *Potassium Nitrate.*

The following preliminary experiments show very well the disastrous effect of the presence of potassium nitrate on the power of carbon-assimilation. In Experiment I, the distilled water contained 1 per cent. NaCl ; in Experiment II, 1 per cent. KNO_3 in addition to 1 per cent. NaCl .

¹ Loew and Bokorny ('87).

² Stange ('92).

³ Jacobi ('99).

Experiment I. January 12, 1900. 1 per cent. NaCl
in distilled water.

Date.	Days.	Amount of Starch.
Jan. 13	1	little ¹
Jan. 17	5	moderate

Experiment II. January 13, 1900. 1 per cent. NaCl
+ 1 per cent. KNO₃ in distilled water.

Date.	Days.	Amount of Starch.
Jan. 16	3	none
Jan. 18	5	the slightest trace

Experiments with different percentages of KNO₃ in distilled water gave the following results. For sake of comparison, a similar series (except that 1.5 per cent. was used instead of 1 per cent.) of percentages of NaCl, made a week later, are also given.

Experiment III. April 13, 1900. *Ulva* in distilled
water containing

Date.	Days.	0.1 % KNO ₃ .	0.5 % KNO ₃ .	1 % KNO ₃ .
Apr. 14	1	a trace	a trace	a trace
Apr. 16	3	a trace	a trace	the slightest trace
Apr. 19	6	a trace	little	the slightest trace

Experiment IV. April 20, 1900. *Ulva* in distilled
water containing

Date.	Days.	0.1 % NaCl.	0.5 % NaCl.	1.5 % NaCl.
Apr. 21	1	a little	a trace	a little
Apr. 23	3	moderate	moderate	moderate
Apr. 26	6	large	moderate	large

¹ The scale of starch-accumulation used will be found fully explained in my former paper.

There was also a marked difference in the condition of the Alga. After six days in the KNO_3 solutions, the seaweed was not far from the point of death, while the Algae in the NaCl solutions were quite healthy. The object of these experiments with distilled water, as in the case of the other nitrates, was to see whether *Ulva* could assimilate in solutions of this salt alone, as it was found to do in the case of sodium chloride. I concluded that this was not the case. The results of Experiments I and II show that the absence of assimilation was not due so much to the absence of certain necessary salts, as to a detrimental effect caused by the presence of the nitrate. With a view to testing this point, I made experiments (as also with other nitrates) in which certain percentages of potassium nitrate were *added to sea water*, on the assumption that if this salt was not harmful to the plant the carbon-assimilation would be normal. A series of solutions were made consisting of sea water to which .5 per cent., 1 per cent., and 2 per cent. KNO_3 was added; and in these *Ulva* was exposed to light. I never obtained from Algae in solutions containing .5 per cent. KNO_3 more than a 'little' starch, from those in 1 per cent. KNO_3 more than a 'trace,' and in 2 per cent. KNO_3 solutions no starch whatever was found. I therefore concluded that the presence of an appreciable amount of KNO_3 in the sea water caused an inhibition of the CO_2 -assimilation.

(B) *Sodium Nitrate.*

As with other nitrates, the proportions of sodium nitrate used were those equivalent to 0.1, 0.5, 1, 2, &c. per cent. KNO_3 , the latter being taken as a standard in all cases. 1 gram KNO_3 is equivalent to .8415 gram NaNO_3 . The following experiment is typical of the effect of this nitrate in sea water:—

Experiment V. June 7, 1900. Sea water + NaNO_3 .

<i>Date.</i>	<i>Days.</i>	<i>Equivalent to 1% KNO_3.</i>	<i>Equivalent to 0.5% KNO_3.</i>	<i>Control sea-water alone.</i>
June 12	5	moderate	not quite moderate	moderate
June 14	7	moderate	moderate	maximum

Similar experiments with distilled water gave results slightly more favourable than those in Experiment III. In all cases in Experiment V, the amount of starch obtained was less than in the control in sea water alone, although after five days in 1 per cent., it was nearly the same. I never obtained a 'large' amount of starch, but on the other hand the Alga continued in good condition for some time, in marked contrast to those in KNO_3 solutions. I concluded that, so far as these experiments went, sodium nitrate was not able to take the place of sodium chloride in regard to CO_2 -assimilation, and that the presence of sodium nitrate in sea water somewhat inhibited the power of CO_2 -assimilation, but not in such a marked degree as in the case of potassium nitrate.

(C) *Magnesium Nitrate.*

This is the nitrate which occurs in very small proportions in sea water: 1 gram KNO_3 is equivalent to 1.465 grams $\text{Mg}(\text{NO}_3)_2$.

Experiment VI. June 7, 1900. Sea water + $\text{Mg}(\text{NO}_3)_2$.

<i>Date.</i>	<i>Days.</i>	<i>Equivalent to 1% KNO_3.</i>	<i>Equivalent to 0.5% KNO_3.</i>	<i>Control sea-water alone.</i>
June 12	5	little	little	moderate
June 14	7	moderate	large	maximum

Experiments in which magnesium nitrate was added to distilled water gave results similar to those with sodium nitrate. I was not able to make as many experiments with this salt as I could have wished, but the results with sea water were, on the whole, very similar to those with NaNO_3 , perhaps a little more favourable, but never quite equal to the control.

(D) *Ammonium Nitrate.*

With this salt experiments were only made with sea water: 1 gram KNO_3 is equivalent to 0.792 gram NH_4NO_3 . In experiments with quantities equivalent to 1 per cent. and

·5 per cent. KNO_3 , no starch whatever was obtained even in a week, or at the most the slightest 'trace.' The control was the same as in experiments with KNO_3 . The Alga became unhealthy, and died in a very short time.

Such conclusions as may, I think, be drawn from these experiments, but which, in some cases, can only be regarded as provisional, are as follows. No solution, containing a moderate percentage of these nitrates *alone*, is at all comparable to one of an equivalent percentage of NaCl , as a medium for normal CO_2 -assimilation. The effect of the presence of 1 per cent. KNO_3 , or its equivalent of the other nitrates experimented with, is in all cases to lower the amount of CO_2 -assimilation as judged by the amount of starch found in the thallus. With magnesium nitrate this is least marked, and next sodium nitrate. Potassium nitrate has a very injurious effect, while ammonium nitrate is absolutely fatal. These results are remarkably in accordance with those of Loew and Bokorny in regard to *Spirogyra*, and also with the conclusions of Stange as to the prejudicial effect of KNO_3 on many fresh-water Algae. In the case of *Ulva*, NaNO_3 was also found to be less injurious than KNO_3 , and the ammonium salt equally fatal, but in the former case there was no sign of any abnormal amount of starch being formed.

These results were, in the main, confirmed at the Bradford meeting of the British Association, where I gave a brief sketch¹ of the chief conclusions to which I had attained. In a paper which followed, by Messrs. Letts and Hawthorne², on the relation of this Alga to the pollution of sea water by sewage, it was stated that no carbohydrates beyond cellulose could be found, on analysis, in specimens growing in the polluted sea water. Dr. Letts said at the time that he was much struck by the absence of all traces of carbohydrates such as starch. These authors have made out a very good case to show that *Ulva* can, under some circumstances, derive its entire nourishment from organic materials, especially from substances rich in combined nitrogen, and without carbon-

¹ Arber ('00), p. 934.

² Letts and Hawthorne ('00), p. 935.

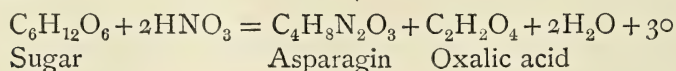
assimilation. There is little doubt, however, that this faculty is only attained by a process of gradual and natural accommodation, the absence of which in the experiments recorded here, I think, fully explains why the Algae did not flourish.

As to the exact cause of the lowering of the CO_2 -assimilation by the presence of these nitrates, it is impossible to offer more than suggestions. It is, however, certain that the result here is not due in any way to plasmolysis. Taking the amount of common salt in sea water as 2.5 per cent., the amount of KNO_3 , which is the isotonic equivalent of 2.5 per cent. NaCl , is about 4.3 per cent. KNO_3 , a larger percentage than was used, and which even then would not have caused plasmolysis. Since plants absorb all substances, essential or injurious, presented to them in a soluble form, it follows that, seeing there is no plasmolysis, the nitrate, with other salts absorbed from sea water, penetrates within the protoplast. This being so, it seems to me that there can be either a physical, or a chemical cause for the effect produced by these nitrates. It is known that many soluble crystalline substances cannot diffuse through certain plasmatic membranes. If this is the case here, the diosmosis of such salts as NaCl , which in *Ulva* would seem to have a peculiar value as absorption-products, would be checked, and consequently an inhibition of the CO_2 -assimilation might take place. On the other hand, this inhibition may be due to a chemical change, which takes place within the plant on the absorption of a considerable quantity of these nitrates, and which may be directly injurious to the protoplast, unless a process of long accommodation has taken place.

With regard to the first of these alternatives, there is no evidence, and I am inclined to favour the chemical theory, to which certain facts would seem to point. Pfeffer¹ says that 'potassium nitrate, when absorbed, is gradually converted into a salt of an organic acid, the traces of nitric acid set free being immediately absorbed by the protoplast.' It is possible that, when a considerable quantity of a nitrate is

¹ Pfeffer ('00), p. 131.

absorbed, a larger amount of free acid would be set free by the decomposition. As such Algae as *Ulva* are well known to be extremely sensitive to acid solutions, which are generally injurious to such plants, the inhibition of the CO_2 -assimilation may be due to a greater amount of acid than usual being set free. The varied degree of inhibition, which different nitrates were found to cause, may conceivably be explained by the metal of certain nitrates having a greater affinity for the organic acid radical. The potassium and ammonium of KNO_3 , and NH_4NO_3 , may be more eager to unite with the oxalic or other organic acid than the sodium or magnesium of NaNO_3 , and $\text{Mg}(\text{NO}_3)_2$, and thus cause a greater inhibition. It was pointed out in a former paper that certain sulphates, especially calcium sulphate, were found to be similarly injurious. Possibly this effect may be explained in the same way, for Schimper¹ has shown that the fate of both nitrates and sulphates is closely analogous, in that they are both reduced. The type of reaction in these cases is—



Schimper has also pointed out that, while potassium oxalate is exceedingly common in plants as the first stage in the formation of calcium oxalate, the presence of sodium oxalate is exceedingly rare. This fact may possibly have some bearing on the assumption suggested here, that the difference in effects caused by potassium and sodium nitrates, is due to a difference of chemical affinity on the part of the metallic radical.

Potassium Phosphate.

With a view to supporting the explanation here suggested to account for the effect of nitrates on *Ulva*, some similar experiments were made with potassium phosphate. The phosphates which could be used for this purpose are very

¹ Schimper ('90).

few, owing to their insolubility or acidity; or because some (sodium phosphate) precipitate in sea water. Four experiments were made in which 1 per cent. potassium-phosphate was added to sea water, and in these starch-free *Ulva* was exposed to light, with a control in sea water under the same conditions. After six days the amount of starch obtained was, in three experiments, 'a trace,' while, in the fourth experiment, a 'little' was obtained. The Alga became unhealthy in a short time. The control continued in good condition, and gave a 'moderate' amount of starch in the same time.

The conclusion was that the presence of an appreciable quantity of potassium phosphate in sea water inhibited the CO_2 -assimilation. Schimper¹ has shown that the fate of phosphates is altogether different from that of nitrates and sulphates. But in the formation of calcium oxalate, which according to Schimper takes place as follows,—potassium phosphate in the presence of oxalic acid forms potassium oxalate, and then potassium oxalate in the presence of an inorganic calcium salt forms calcium oxalate,—it is clear that, in the first step at least, phosphoric acid is set free. Hence from the standpoint adopted here, the amount of free acid, the result with phosphates should be similar to that with nitrates and sulphates, as indeed it was found to be.

Phosphorus, Iodine, Iron.

I have now concluded experiments on what I have termed the *principal salts* of sea water, using that term only in the sense of forming the greater percentage of that substance. Other elements, such as iron and phosphorus, are equally essential to the plant, but are remarkable as occurring only in the minutest traces in sea water. Although no experiments, with the exception of those on potassium phosphate, were made with these bodies, it may be of interest briefly to point out a fact too generally overlooked, namely, how minute the traces of these elements in sea water really are.

¹ Schimper ('90).

	Percentage in sea-water.	Percentage in total ash of various Fucaceae ¹ .
Iron	0.0005	0.29–0.34
Combined nitrogen	0.0002	
Iodine, less than .	0.00001	0.31–1.13
Phosphorus	—	1.36–4.4

With regard to phosphorus, no chemical analysis of sea water contains any estimation whatever of that substance; the trace being infinitely small². Noll³ has drawn special attention to this in a paper on the culture of marine Algae. The same author⁴ regards iodine, which occurs in an almost equally small proportion, as not indispensable to Algae. The necessity of iron was first pointed out by Gris⁵ in 1843, and his are probably among the earliest observations, which showed that an inhibition of the carbon-assimilation in a plant can be caused by the absence of an essential inorganic salt. Molisch⁶ has found, and it is generally admitted, that the molecule of chlorophyll does not contain iron, and it is therefore highly probable that iron is essential to the metabolism of the chloroplast. It would seem, therefore, that in sea water we have certain essential elements, in what is probably the smallest degree of concentration ever made use of in nature by plants. A better illustration of the selective capacity of the plant, and its power of absorbing substances in direct proportion to the amount necessary for metabolism, and in altogether disproportionate amount to the ratio of the substances in a given volume of the medium, would be hard to find.

THE STARCH-ACCUMULATION IN MARINE ALGAE.

One point, which has been very prominent throughout these experiments, is the large accumulation of starch which occurs in these Algae, and the very slow rate of translocation.

¹ Goedeckens, vide Pfeffer ('00), p. 128.

² Noll ('92), pp. 282–3.

³ Gris ('44).

⁴ Voelcker ('50), pp. 346–7.

⁵ Noll ('92), p. 285.

⁶ Molisch ('92).

This is marked even in *Ulva*, and as I showed in a previous paper, formed one of the chief difficulties of the work. The Alga had to be darkened for several weeks before becoming starch-free. At the conclusion of the work on *Ulva*, I was very anxious to make similar observations on other marine Chlorophyceae. Unfortunately very few members of this group are at all suitable for such a purpose, and in none is the form of the thallus so favourable for observation as in *Ulva*. *Enteromorpha intestinalis*, Link, was tried, but abandoned as I was unable, even after weeks or months of darkening, to get all the starch out. Not having the time at my disposal to attempt a direct estimation of the amount of carbon assimilated,—a problem seemingly of great difficulty when the plant is immersed in a liquid,—I determined to try *Cladophora rupestris*, Kg. Material, which was very kindly sent me by Professor Phillips, was completely darkened in sea water in the laboratory, but after two months the Alga still contained starch and was becoming so unhealthy that, although another and equally unsuccessful attempt was made in very diffuse light, the work had to be abandoned. It had occurred to me that want of oxygen, or insufficiency of salts in a limited quantity of sea-water, might account for this. Pennington¹, in regard to similar difficulties with the destarching of *Spirogyra*, found that the former played a very important part in the process. I therefore arranged, through the courtesy of the Director, for the destarching to be carried out at the Marine Biological Laboratory at Plymouth. A large tank there was almost completely darkened, and through it a supply of sea-water was pumped continuously, the inflow impinging sharply on the surface of the water in the tank, and thus ensuring sufficient saturation with air. The tank was stocked early in January with *Cladophora* which I obtained from the neighbouring coast. After five months' darkening, while the Alga continued healthy, and in good condition, it still contained starch. In a few experiments which I made with the partially destarched Alga, by exposing it to light in sea water, I found

¹ Pennington ('97).

that the rate of accumulation was correspondingly long. It was also found to be by no means so easy as in *Ulva* to judge of the amount of starch in the thallus, even roughly, and for these reasons the work was finally abandoned. *Cladophora rupestris* is undoubtedly not an obligate Halophyte, and it is possible that in such Algae the necessity for sodium chloride, which I found in the case of *Ulva* to be so important for the maintenance of carbon-assimilation, is not so marked, and that the plant can continue to make use of other salts, more especially those of fresh water, for such a purpose. This would seem to be the real distinction between an obligate and nonobligate Halophyte, rather than any insufficient selective power on the part of the former. Unfortunately, however, for the reasons just described, I am not able to add anything for or against this supposition.

In conclusion I have to again express my thanks to Mr. Darwin for the interest he has taken in the work and the help which he has at all times placed at my disposal. I have also to express my indebtedness to the Director, and to Mr. Garstang, of the Marine Biological Laboratory at Plymouth, for the courtesy with which every facility was afforded for the work which I carried on there.

CONCLUSIONS.

(1) The addition of a nitrate to sea water causes an inhibition of the carbon-assimilation.

(2) With ammonium nitrate, the inhibition is very marked, and the presence of this salt is quickly fatal.

(3) Potassium nitrate causes a more marked inhibition than sodium nitrate.

(4) Magnesium nitrate, the nitrate occurring in sea water, causes the least marked inhibition.

(5) Potassium phosphate added to sea water in an appreciable percentage, causes a considerable inhibition.

(6) In the thallus of *Ulva*, *Enteromorpha* and *Cladophora*, there is a marked storage of starch, and a very slow rate of translocation.

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On *Diplodia cacaoicola*, P. Henn. ;
a parasitic Fungus on Sugar-Cane and Cacao
in the West Indies.

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With Plate XXXVII.

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WHILE engaged from time to time since December, 1899, in a search, which has so far been unsuccessful, for the perithecia¹ of *Trichosphaeria Sacchari* (Masse), among diseased sugar-canes in Barbadoes and elsewhere, a Fungus was very commonly met with which was distinguished by having brown elliptical bi-septate spores developed in pycnidia just underneath the rind of the cane, and which ruptured the surface when ripe. To the naked eye there is little difference between this Fungus and the *Melanconium* stage of the 'rind Fungus' described by Masse, and it was observed that when this new form predominated on diseased canes it was locally regarded as 'rind Fungus.' Indeed in the West Indies at the present time it is customary to regard all cane diseases as either 'rind' or 'root' disease, and many are of opinion that both these diseases are caused by one Fungus. It is very probable, however, that there are several rind, leaf, and leaf-sheath

¹ On *Trichosphaeria Sacchari*. Ann. Bot., vol. vii, p. 128, 1893.

[Annals of Botany, Vol. XV. No. LX. December, 1901.]

diseases, and that 'root' disease is nothing more than a general term covering several apparently distinct maladies.

During the early part of last year cultivation-experiments were performed with the Fungus, and no difficulty was experienced in obtaining hanging-drop cultures containing a single spore. The germination of the spores was observed in several culture-media, and the Fungus was grown in flasks, in tubes, and on sterilized wood. At this time frequent absence from the laboratory and pressure of other duties prevented a complete study of the Fungus being made, and it was not till last December, when an outbreak of what was described as 'rind' Fungus occurred in Demerara and diseased canes were forwarded to the Department of Agriculture by G. S. Jenman, the Government botanist of British Guiana, that the study of this form was resumed.

It was then found that the predominant Fungus on these canes was identical with the pycnidial form noted earlier in Barbadoes, and since many of the canes appeared to contain this Fungus alone, experiments which are described below were undertaken to follow the development of this form under artificial conditions, and to prepare pure cultures.

About this time the cacao planters in Grenada had their attention directed to the diseases of cacao by the spread of 'thrips,' which did some slight damage to the pods and the young leaves of the trees. It was observed that several trees on which 'thrips' had been seen died off rather rapidly, and considerable alarm was caused by the belief that these insects might destroy the cacao industry in that island. An investigation on the spot, however, showed that it was highly probable that the trees believed to be dying through the attack of the thrips were really being killed by Fungi, and that the insects were not more numerous on the diseased trees than on others which were quite healthy. Portions of the branches of such trees were collected and sent to Barbadoes for examination, when it was found that in all cases they contained the same Fungus, and that this was apparently identical with that found on the diseased sugar-canes from

Demerara. Experiments, which are described below, were undertaken to follow the development of this cacao Fungus, starting from a single spore. The behaviour of the two forms was therefore studied side by side, and the results are given in the following.

In February and March of the present year I was requested by Dr. Morris, the Imperial Commissioner of Agriculture for the West Indies to visit Grenada and to report on the nature and extent of the damage done to cacao trees by fungoid diseases. While engaged on this mission I found that in addition to its occurrence on cacao trees, the Fungus was common on growing cacao pods, where it was apparently the cause of a rather serious disease. Infection-experiments with pure cultivations of the Fungi found on canes and on the cacao tree, on their respective host-plants, and also cross-infection experiments were carried out during this time, and are described below. Later in the present year a Fungus on cacao was noted in St. Lucia, St. Vincent, and Dominica, which proved to be identical with that found in Grenada.

THE SUGAR-CANE FUNGUS.

The external appearance of a sugar-cane in which this Fungus occurs is not unlike that of a cane attacked by the *Melanconium* stage of *Trichosphaeria Sacchari*. The rind is ruptured by the growth of dark bodies underneath, which are arranged in more or less vertical lines (Pl. XXXVII, Fig. 1). Transverse sections through these black areas show that the disturbance is caused by the development of colonies of true pycnidia formed just beneath the rind, and which are in connexion with a dark-brown septate-branched mycelium abundant in the tissues of the cane (Fig. 2). The wall of the pycnidium is seen to be made up of two distinct layers, an outer *peridium* made up of dark-coloured, thick-walled hyphae forming a definite tissue, and an inner hymenium of thin-walled colourless cells, charged with granular contents, which are no doubt destined for spore-formation. In the cavity of the pycnidium short conidiophores, 20-40 μ in length, bearing

elliptically shaped spores in all stages of development, and numerous paraphyses, are to be seen. The detailed structure of a pycnidium seen under the high power is shown in Fig. 3. It agrees closely with that described by Bauke¹ in the case of a *Diplodia* on *Cornus sanguinea* and figured in Zopf².

In cases when pieces of diseased cane, on which this Fungus was found, were placed in a moist chamber, it was observed that there was a considerable development of hair-like processes on the walls and round the opening of the pycnidium, giving the colonies a furry appearance which was never noted in the cane in ordinary circumstances.

The spores are liberated from the mature pycnidium by means of a circular ostiole at the apex (Fig. 4), and are at first greyish and unicellular. In a short time, however, the exospore rapidly darkens and becomes dark brown, while at the same time a transverse wall is formed across the equator of the spore. The mature spores are very constant in size and measure $20 \times 10 \mu$.

The development of the Fungus was next studied, starting from a single spore. No difficulty was experienced in obtaining hanging-drops containing a single spore by the method described by Marshall Ward³ in his investigation on the ginger-beer plant. The spores germinated quite readily in such media as 15 to 20 per cent. gelatine raisin-extract or cane-juice, but the best results were obtained in the following food-material which is referred to later as the cane-extract medium:—

Cane sugar	.	.	.	5 grams.
Gelatine	.	.	.	15 „
Tartaric acid	.	.	.	1 „
Peptone	.	.	.	5 „
Cane-extract	.	.	.	100 c. c.

The cane-extract was obtained by soaking small pieces

¹ Beitr. z. Kenntniss der Pycnidien, Nova Acta, Bd. xxxviii, No. 5.

² Zopf, Die Pilze, p. 60.

³ The Ginger-beer Plant and the organisms composing it. Phil. Trans. B., vol. 183, pp. 130-2, 1892.

of ripe sugar-cane in distilled water for half an hour, boiling, and then filtering. It was found that the addition of the small quantity of tartaric acid was sufficient to check the development of Bacteria in the hanging-drops, and to permit of the mycelium developing in such a way as to obtain pure cultures from the drops.

The ripe spores germinate in from three to six hours after sowing by sending out a colourless hypha from one of the compartments of the spore, which contains finely granular protoplasm (Fig. 4). The hypha grows in length, slowly at first, afterwards much more rapidly, and then branches: about twenty-four hours after sowing septa begin to appear.

In cases where spores are sown which are still colourless and have not yet developed the transverse wall, germination is more rapid than in the case of ripe spores, and takes place in less than two hours after sowing. The earlier stages in the germination of such a spore are shown in Fig. 5.

Two days after sowing, fusion of hyphae was common in the drops. A young hypha approaches very close to one somewhat older, and the advancing tip of the young hypha curves round to meet a short branch pushed out by the older filament, and fusion rapidly takes place (Fig. 6). Where hyphae have crossed in the older regions of the colony a short process is sent out near the crossing from each of the hyphae, and these fuse.

About this time the hyphae commence to grow down into the moist air below the drop, and their development goes on until the mycelium reaches the water on the floor of the culture-chamber, where considerable subsequent growth takes place. The appearance of these positively hydrotropic columns of mycelium reminds one somewhat of the hanging roots of the banyan: the appearance is shown in Fig. 7. From the point of view of pure cultures this aërial development is an obvious advantage, since further cultures which proved to be free from Bacteria were obtained by infecting flasks of sterile food-material, and tubes containing sterile wood, with portions of this mycelium developed from a single spore.

When three days old the mycelium showed signs of darkening in hue, and this tendency increased till the colour was dark olive-green when seven days old. During this colour-development oil-drops appeared in the hyphae, the contents of which gradually collected into certain portions of the mycelium, leaving the rest empty (Fig. 8).

No further developments were observed in hanging-drops, except that the thick-walled hyphae became covered with a dark-brown resin-like covering, like that mentioned by Bauke in the case of a *Diplodia*¹. The old mycelium in the cane near mature pycnidia shows this brown covering also.

In plate-cultivation made with spores from a diseased cane by the dilution-method, using the cane-extract medium, pure cultures were obtained containing only one colony, which in three days developed a copious greyish mycelium, and this, in spite of Bacteria, covered the plate and grew into the air, forming a dense pile about a quarter of an inch in height. When four days old, dark circular hairy bodies were noted, which proved to be pycnidia containing the characteristic spores of the Fungus when examined on the ninth day.

When grown in flasks or tubes of the cane-extract food-material the development is very similar to that on plates, except that when the cultures are free from Bacteria the mycelial development is more luxuriant and the surface of the food-material becomes covered with a dense felted mass of mycelium about half an inch thick, which soon turns black, and in which numerous colonies of pycnidia are embedded.

Such luxuriant development, however, is not so satisfactory as that obtained on sterile oak- or cacao-wood infected with some of the aërial mycelium from hanging-drops containing a single spore. Such cultures, on account of their slow development, were found to be of service in maintaining a stock of fairly fresh pure-cultures during the progress of the investigation, and for use in the infection-experiments. Pycnidial formation does not begin in this case before from six to ten days, and spores cannot be usually detected in

¹ De Bary, Comparative Morphology and Biology of the Fungi, p. 10.

them in less than two or three weeks. In one case, however, a pycnidium was examined from a ten-days-old culture on oak-wood, and stages were observed in spore-formation. The spores are liberated after vacuoles have appeared in the conidiophores. A portion of the hymenium of this artificially grown pycnidium showing spores and paraphyses, is also represented in Fig. 9.

No further developments were noted in the various cultures, which were kept under observation for some months.

It now became necessary to carry out infection-experiments with pure cultivations of the Fungus, and to determine to what extent it can behave as a parasite. Fully developed and extremely vigorous seedling-canes of the variety known as 'Barbadoes 347' or 'B. 347' were selected for the experiment. Incisions were made with a sterile knife in the centre of one of the internodes, about halfway up the stem of the six canes selected for the experiment, after cleaning the internode carefully with alcohol. A small chamber was cut under the rind, into which portions of mycelium from a seven-days-old pure culture of the Fungus in the cane-extract medium were introduced, together with some of the food-material. The wounds were then bound up with budding-tape. Four canes were infected with mycelium, while the other two were treated similarly except that no mycelium was introduced and thus served as control canes. The result was most marked. In four days all four inoculations of the infected canes had taken, and the mycelium could be traced in the parenchyma of the internode for about two inches in all directions round the point of infection. In two cases pycnidia had formed under the rind near the point of infection. The control experiments gave negative results, and no reddening of the tissues was observed in the four infected canes.

This result entirely confirmed previous infection-experiments made on sugar-canes in Barbadoes in the early part of 1900, when the Fungus was found to behave parasitically.

THE CACAO FUNGUS.

The Fungus found on the pods and branches of the cacao tree agrees, as far as the characters of the pycnidia and spores go, exactly with the form described above on the sugar-cane. In the case of the pods the pycnidia are often formed singly just under the epidermis, and the latter is ruptured by the growth of these structures. In diseased branches, or on the trunk of the tree, the pycnidia occur usually in colonies just under the bark. The appearance of a branch attacked by the Fungus, which has been kept in a moist chamber a short time, is shown in Fig. 10, and a transverse section through one of the colonies is given in Fig. 11.

Some general indication of the appearance of cacao trees and pods apparently attacked by this Fungus may be of interest. It is quite common in Grenada to see cacao trees dying back to a slight extent at the extremities of the branches, a phenomenon probably due to poverty of soil, wind, drought, or defective root-action, or perhaps to a combination of these causes. In all such cases there is a sharp line of demarcation between the dead and living tissues, and although several Ascomycetes are to be found in the dead wood, they appear to be purely saprophytic in character. In many cases, however, dying back goes on to a very great extent, extending to the larger branches and the trunk, and, in spite of the production of suckers at the base, the trees are often killed outright. In such cases there is no boundary line between dead and living tissues, but an intermediate zone, often as much as two feet in length, always occurs between the obviously dead and living tissue. Mycelium can be easily detected in the young wood in this transition-region, and the pycnidia referred to above are to be found under the bark. The mycelium can be detected in the wood at some distance in advance of the point at which it seems to end in the bast, a point which seems to indicate the saprophytic origin of the Fungus. The mycelium makes its way in the wood from element to element by means of the pits in the walls of the

vessels and cells, and where the hyphae have commenced to darken in colour this point can be determined without staining (Fig. 12).

In cases where pods are apparently attacked by the Fungus this is very common near the 'breaking-grounds,' where the 'beans' are extracted by the pickers, and where it is the custom to leave the empty husks on the ground in heaps. The husks speedily become covered with the spores of the Fungus, as this form lives on them as a saprophyte. The rind of the pod turns brown and mycelium soon spreads to the mucilage surrounding the seeds, completely destroying the pod and its contents, usually in from six to ten days. The diseased areas commence as a brown spot, as a general rule either at the free end of the pod, or in the groove round the insertion of the stalk, or at the point where the pod comes in contact with the branch. These places are those which are liable to be moist long after the rest of the pod is dry, and indicate the probability that infection may here be effected by spores without any previous wounding.

The artificial cultivation of the Fungus was carried out in an exactly similar manner to that employed in the case of the sugar-cane Fungus described above, and as far as possible similar cultures of the two forms were made and examined at the same time.

Stages in the germination of the spore are shown in Fig. 13. The germ-tube grows out into a long hypha, at first slowly, but afterwards much more rapidly, and extensive branching eventually takes place. Septation of the hyphae was not noted before twenty hours after sowing, and after two days fusions of the hyphae were common: about the same time the mycelium commenced to grow down towards the water on the floor of the moist chamber, reaching it four days after sowing (Fig. 7). When three days old the mycelium gradually changed colour, passing through various shades from light yellow to olive green, and at the same time the hyphal contents began to aggregate in certain portions of the mycelium, leaving the rest empty. Oil drops also made their appear-

ance. The appearance of the mycelium, when eight days old, closely resembles that shown in Fig. 8. No further development was noted in hanging-drops, except that the thick-walled oil-containing hyphae became dark brown three weeks after sowing, and appeared identical with the old mycelium described above in the case of the cane Fungus. As in the sugar-cane Fungus, advantage was taken of the aërial development of mycelium in single-spore hanging-drops to prepare cultures free from Bacteria.

In plate-cultures in the cane-extract medium a copious development of greyish mycelium was obtained on the third day, which formed a dense velvety pile, a quarter of an inch in height, on the surface of the gelatine, and in which dark bodies could be detected with the naked eye. When examined on the sixth day these dark bodies were found to be pycnidia in which paraphyses and spore-formation could be detected, after the manner indicated in Fig. 9.

On sterilized cacao- and oak-wood small dark bodies were noted in nine days, and these when twenty days old proved to be the pycnidia of the Fungus.

It will be seen therefore that the development, under artificial conditions, of the Fungus of the cacao tree corresponds exactly in all its details with that found on the sugar-cane, so that morphologically regarded the two forms are identical.

It was now necessary to perform infection-experiments on cacao trees and pods, with pure cultivations of the Fungus. These were as follows:—

1. On cacao pods:

(a) Two nearly ripe pods were selected for the experiment, and were washed with alcoholic corrosive sublimate, and at the points where incisions were to be made small cavities were made in the rind by lifting the surface and cutting out a small portion of the tissues underneath. Into one of these chambers actively growing mycelium, three days old and from a pure culture, was introduced and the pod was bound up with budding-tape. The other pod was treated in a similar way, except that no mycelium was introduced and thus served as

a control. Five days afterwards, about a quarter of the surface of the infected pod had turned brown, and in eight days after infection the whole of the surface was deep brown, and there was a considerable development of pycnidia for some distance round the point of infection. The protrusion of the spores as a greyish 'tendrils,' visible to the naked eye, from the ostiole of the pycnidium, and their gradual darkening were beautifully shown on this pod. Near the point of infection the spores were visible as a black dust on the surface of the pod, and in most cases the 'tendrils' had broken down into their constituent spores, each showing the transverse wall and the dark-brown colouration under the microscope. Further away the colour of the spores became lighter and 'tendrils' were more numerous. These were composed of spores loosely cemented together, in which the transverse wall had not yet appeared. The control pod in this experiment showed no infection.

(b) The above preliminary experiment was repeated, and in this case two nearly ripe pods were infected with mycelium, and a third was used as a control. Distinct infection took place in three days, while the control gave negative results.

(c) Next, four half-grown pods were selected for experiment, in order to determine whether the spread of the Fungus is as rapid here as in nearly ripe pods. In each case small cavities were made in the rind, and in the first pod ripe spores from the infected pod in experiment (a) above were placed in the cavity. In the second pod a portion of the rind containing growing mycelium from the infected pod (a) above was introduced, and in the third pod actively growing mycelium from a pure culture was used for infection. The fourth pod served as a control. Seven days afterwards distinct infection was noted in the first pod, the rind having turned brown about a quarter of an inch all round the cavity, and in the discoloured tissue mycelium was extremely abundant. In the second pod infection had proceeded further, about one square inch of the surface being attacked. In the third pod about six square inches of the surface was decayed, and

it was found that the mycelium had penetrated to the mucilage surrounding the seeds and had completely invaded the interior of the pod. Numerous pycnidia were observed under the epidermis near the point of infection. The control pod gave negative results. This experiment is of some interest as it throws light on the steps by which a saprophytic form may gradually become parasitic, and confirms previous observations on the influence of a nutritious food-material in increasing the activity of a Fungus.

(d) Next, a preliminary experiment was performed on two nearly ripe cacao-pods, in order to determine whether infection could be produced by spores without previously wounding the rind. A drop of sterile water containing spores was placed on the surface of the pod, and was covered by a small glass cell which was sealed on to the rind by means of budding wax. The cell was covered with a dark bandage to shield the spores from direct sunlight. Seven days afterwards the spores had developed a mycelium on the pod, but penetration of the intact rind by mycelium was not noted in either case. Unfortunately time did not permit of carrying out further experiments to settle this point definitely; but a consideration of natural infections seems to indicate that pods at any rate are capable of being infected by germinating spores directly. If this were not the case it is difficult to explain why infection almost always begins at those points on the pod which are moist for the longest time, viz. the free end of the pod, the groove at the insertion of the stalk, and the place where pods come in contact with the branches.

2. On the cacao trees:

In the infection-experiments performed on the cacao tree itself, a small portion of the outer dry bark was carefully removed and the exposed bast washed with sterile water. A small chamber was made by raising the bark and cutting out a small portion of the bast down to the cambium. After introducing the infecting material, the bark-lid of this chamber was depressed and the whole covered with a water-

tight bandage of budding-tape. The control plants were treated in the same way, except that no mycelium or spores were purposely introduced into the cavity.

(a) Two branches of a healthy cacao tree were selected for the preliminary experiment. In one cavity actively growing mycelium in the cane-extract medium was introduced, while the other branch was used as a control. Eight days after infection the Fungus had killed the bast up to about eight inches above and below the chamber, at which point the branch was nearly ringed. The mycelium could be traced more than a foot above and below the chamber, both in the bast and in the wood, and to a considerable depth in the latter. Numerous pycnidia were developed under the attacked bark, some of which were liberating their spores. The control showed no infection.

(b) Eight healthy cacao plants about eight months old, growing in bamboo pots, were next selected for an experiment. Nos. 1 and 2 were infected with spores taken from a pod attacked by the Fungus, 3 and 4 with portions of the diseased rind of a pod containing actively growing mycelium, 4 and 5 with vigorous mycelium from a pure culture in cane-extract and 7 and 8 were control plants. After binding up the wounds the plants were placed in the shade and watered daily. Eight days afterwards it was found that infection had taken place in all the plants from 1 to 6, while the control plants showed no infection. There was scarcely any difference in the amount of infection when spores or diseased cacao-pod rind were used, except that plant No. 1 was killed outright by the Fungus. When culture-mycelium was used infection was more extensive, one of the plants being killed while the other was evidently dying. In each case (5 and 6) pycnidia were formed under the rind.

(c) Four vigorous young trees about eighteen months old were now selected. The first was used as a control, the second was infected with spores, the third with a portion of a diseased pod taken from near the still healthy tissue, and the fourth with pure culture-mycelium. Eight days

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afterwards distinct infection was noted in No. 2, while in Nos. 3 and 4 the trees were nearly ringed near the infection-chamber, and the mycelium could be traced in the bast and wood as far as six inches above and below this point. Pycnidia were noted under the bark near the chamber. The control tree showed no infection.

(d) Attempts to induce infection by spores growing in water on the bark, in a manner similar to that used in experiment 1 (b) above, failed. The spores germinated, but I could not detect any penetration of the living tissues by the hyphae.

These infection-experiments show that the Fungus can behave as a parasite towards cacao pods, and is a dangerous wound-parasite of the cacao tree itself. The nature of one of the tree-diseases in Grenada, and of an important pod-disease, is therefore placed beyond doubt.

ONE FUNGUS OR TWO?

Up to this point it had been demonstrated that the Fungi on sugar-cane and on the cacao tree, as dealt with above, are both capable of pronounced parasitism on their respective hosts, and in addition are morphologically identical. It became therefore an interesting question to determine whether or not parasitism on these widely different hosts had become so closely adapted to these hosts that cross-infection was impossible. To answer this question the following series of infection-experiments was carried out, in which an attempt was made to infect cacao trees and pods with pure cultures of the sugar-cane Fungus, and also to try the effect of the cacao Fungus on the sugar-cane.

Infection of Cacao Tree and Cacao Pods with the Sugar-Cane Fungus.

(a) Two branches of a healthy cacao tree were infected with pure culture-mycelium of the sugar-cane Fungus, while a third was used as a control. Four days afterwards the

invading mycelium could be distinguished both in the wood and bast as far as six inches from the point of infection.

Cultivations made by infecting sterile tubes of food-material with small portions of tissue taken from the bast at this distance from the wound, developed the characteristic pycnidia and spores of the Fungus. The control experiment showed no infection.

(b) Three green pods were selected for this experiment. In a cavity of two of these, mycelium of the Fungus, seven days old, was introduced, while the third was used as a control. In three days the inoculated pods showed that the mycelium had spread through about a quarter of the pod, and cultures of a portion of the diseased tissue, taken as far as possible from the point of infection, developed the characteristic organs of fructification of the Fungus. No infection was detected in the control pod.

Infection of the Sugar-Cane with the Cacao Fungus.

Three healthy B 347 canes were selected. In two of them pure culture-mycelium was introduced into a small cavity made under the rind, and the third served as a control. In eighteen days the mycelium had spread into the tissues of the internodes above and below that in which the infecting chambers were cut, and the characteristic pycnidia of the Fungus were formed in large numbers under the rind in the internode in which infection was made. No infection was detected in the control cane.

These cross-infection experiments leave no doubt that the two Fungi are identical, and that the Fungus has not yet adapted itself to its host closely enough to give rise to two physiological species.

PROPHYLAXIS.

In dealing with the remedial measures which might be adopted on estates to check the ravages of this Fungus, it is well to remember that it readily lives as a saprophyte on old cacao-pod husks, and on the dead wood which is usually

left under the trees. Great care should therefore be taken to bury all the old pods, and to collect and burn all diseased pods noticed on the trees, together with all the dead wood found in the plantations. The use of tar after pruning would also to a great extent check infection of growing trees. In sugar-cane cultivation, if the diseased canes were regularly collected and burnt at the reaping season there would be less chance of infecting the next crop. The cacao planters in the West Indies are now beginning to adopt these measures, with the result that the pod-disease described in this paper is much rarer on such estates than on others where no precautions are taken.

SYSTEMATIC POSITION OF THE FUNGUS.

In the absence of any higher fructification than the pycnidia described above, the Fungus must be referred to the Fungi Imperfecti, and, on account of the character of the pycnidia and the brown two-celled spores, falls into the division *Sphaeroidaceae-Phaeodidymae* of the *Sphaeropsidales*, which includes the genus *Diplodia* and its allies. Specimens of the Fungus were referred to Kew, where it was determined by Massee as *Diplodia cacaoicola*, P. Henn., a form occurring on dead cacao branches in the Cameroons¹. In the classification given by Lindau in 'Die natürlichen Pflanzenfamilien'² mention is made of an allied Fungus, *Botryodiplodia Theobromae*, Pat., as occurring on cacao fruits in Ecuador. Lecomte and Chalot³, in their book on Cacao, in discussing the South American diseases known as *Mancha*⁴, state that Patouillard found this Fungus on diseased cacao-pods from Ecuador, and that probably one of the forms of *Mancha*⁵, in which the rind turns brown and the pods dry up, may be due to this Fungus.

¹ Engler's Jahrb., Bd. xxii, p. 80, 1895.

² Engler and Prantl, Die natürlichen Pflanzenfamilien: Fungi, I. Theil, 1. Abteilung, p. 372.

³ Lecomte and Chalot, Le Cacoyer et sa culture.

⁴ Sodiro, Observaciones sobre la enfermedad del Cacao 'Mamada,' &c. Quito, 1892.

⁵ De Lagerheim, Pflanzenpathologische Mittheilungen aus Ecuador. 1892.

On referring to Lindau's classification of the *Sphaerioidaceae-Phaeodidymae* given in Engler and Prantl, it appears that the group is divided up according to the occurrence of the pycnidia singly or in colonies, and according to the presence or absence of a stroma.

In *Diplodia* the pycnidia are free from each other, occurring singly, and there is no stroma. In *Botryodiplodia* they are arranged in colonies and a stroma is present. In considering the behaviour of the Fungus under discussion under artificial conditions and on the host-plants, it will be seen that there is great variation in the arrangement of the pycnidia, as they sometimes occur alone, at other times in colonies. There is, besides, a good deal of variation in the amount and arrangement of the hyphae surrounding the pycnidia. In some cases the latter appear to be embedded in a stroma, in others to stand in a web of hyphae. It would therefore appear likely that the Fungus on cacao pods in Ecuador is identical with that which is so common in the West Indies.

The Fungus does not appear to have been noted in Java on the sugar-cane, as no mention is made of it in Wakker and Went's¹ book on the diseases of the cane or in Krüger's 'Das Zuckerrohr.'

It appears, however, to have been noted previously in the West Indies in 1878, when specimens were sent to Kew for examination. Some confusion in the literature seems to have arisen from these specimens, as will be seen by the following note by Massee in the Kew Bulletin²:—

'Specimens of diseased sugar-cane were sent to Kew in 1878 from Porto Rico for investigation. These were submitted to the Rev. M. J. Berkeley, who gave the MS. name of *Darluka melaspora* to the Fungus present on the canes. The Fungus was afterwards very briefly described under Berkeley's name by Cooke in 'Nuovo Giornale Bot.,' vol. x, p. 26 (1878), where the locality was incorrectly given as Australia, instead of Porto Rico. Saccardo has added to

¹ Wakker and Went, *De Ziekten van het Suikerriet op Java*. Leyden, 1898.

² Kew Bulletin, p. 86, 1895.

the confusion by changing the name to *Coniothyrium melasporum*, and by quoting Cooke's diagnosis incorrectly in Syll. Fung., vol. iii, No. 1799.

'Finally, Prillieux and Delacroix, in their paper on Sugar-cane Diseases (Bull. Soc. Mycol. de France, tom. xi, p. 75, 1895), have fallen into the error of considering the *Melanconium*-stage of *Trichosphaeria Sacchari* (Masse) to be synonymous with *Coniothyrium Melasporum*, (Berk.) Sacc. Examination of Berkeley's type specimen shows that the Fungus is a *Diplodia*.'

There can hardly be any doubt that this *Diplodia* is identical with the species discussed in this paper.

BARBADOES.

August 2, 1901.

EXPLANATION OF FIGURES IN PLATE XXXVII.

Illustrating Mr. Howard's paper on *Diplodia cacaoicola*.

Fig. 1. Portion of the stem of a sugar-cane, showing the pycnidial colonies of the Fungus breaking through the rind. Nat. size.

Fig. 2. Transverse section of the sugar-cane stem, showing pycnidia. Zeiss. a a.

Fig. 3. Transverse section of a pycnidium breaking through the rind of the sugar-cane. Zeiss. D D. Drawn with the help of a *camera lucida*.

Fig. 4. Stages in the germination of a spore obtained from a pycnidium on a diseased sugar-cane. The sowing was made at 9.30 a.m. Dec. 22, *a*, at 3 p.m.; *b*, at 3.55 p.m.; and *c*, at 5 p.m. the same day. Temp. 27-29° C. At 8 a.m., Dec. 23, a hypha had developed from the other half of the spore, but the single-spore colony was too complex to draw. $\times 375$.

Fig. 5. Stages in the germination of a young spore in which the transverse septum has not yet appeared. The spore was obtained from a pycnidium from a diseased sugar-cane, and the sowing was made at 9.30 a.m., Dec. 22; *a*, at 11.30 a.m.; *b*, at 12 noon; *c*, at 1.30 p.m.; and *d*, at 4 p.m. the same day. Temp. 27-29° C. *a*, *b*, *c* $\times 375$. *d* $\times 60$.

Fig. 6. Stages in the fusion of hyphae in a hanging-drop, two days after sowing: *a*, at 2.5 p.m.; *b*, at 4.45 p.m.; and *c*, at 5.0 p.m., Dec. 24. Fusion was complete at 5.30 p.m. the same day. $\times 375$.

Fig. 7. Culture-tube, showing the passage of mycelium from the hanging-drop to the water on the floor of the moist chamber in a four-days-old culture of a spore obtained from a diseased cane. A similar development was noted in cultures of spores obtained from a diseased cacao branch. Nat. size.

Fig. 8. Mycelium from a seven-days-old hanging-drop culture in a resting condition. The darker hyphae are olive green in colour, and contain numerous oil-drops; the others are empty. $\times 375$.

Fig. 9. *a*. Stages in the formation of spores in a pycnidium from a ten-days-old culture on oak-wood.

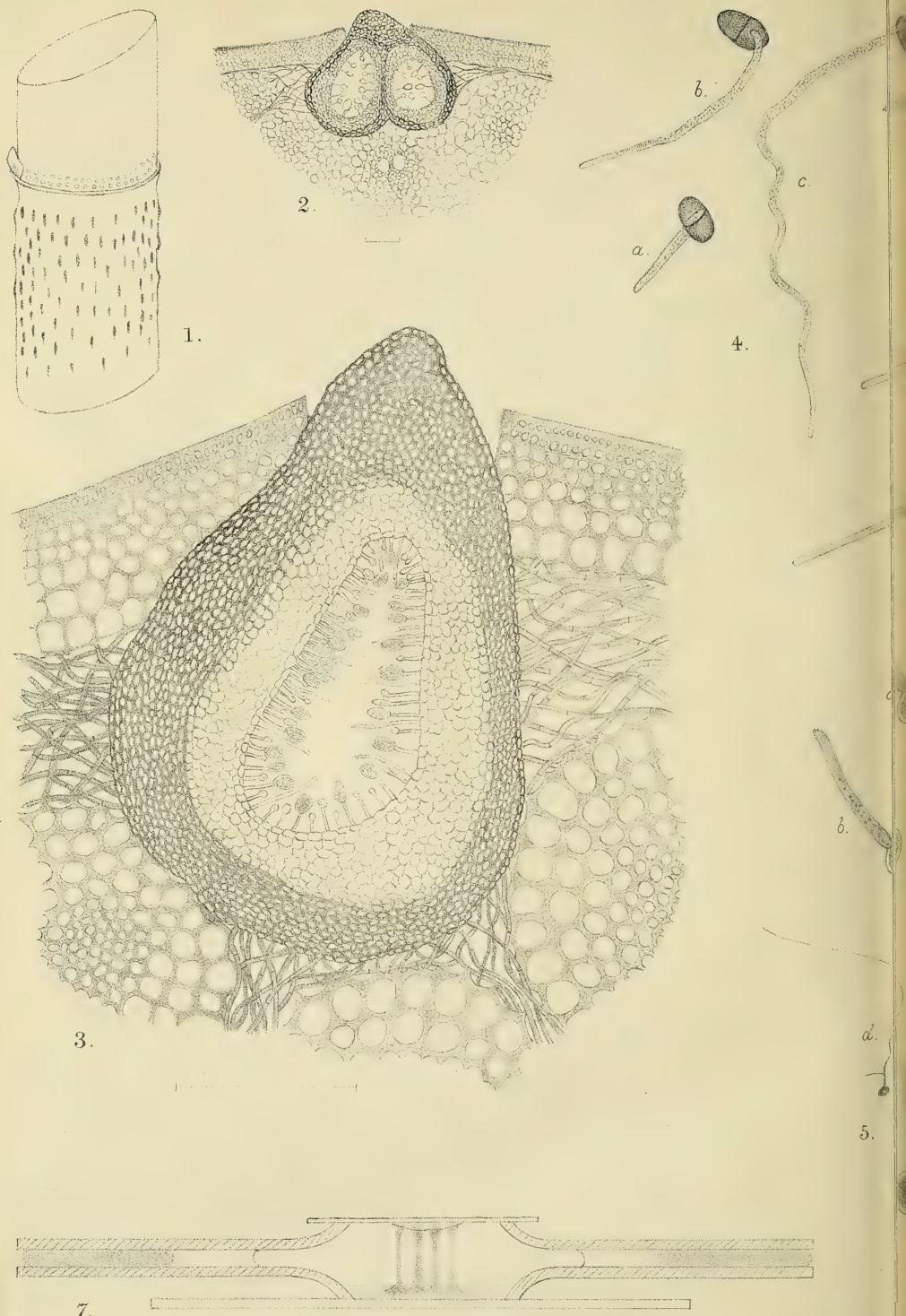
b. A portion of the hymenium of a pycnidium from the same culture, showing conidia and paraphyses. (The parent spore of this culture was obtained from a sugar-cane.) $\times 375$.

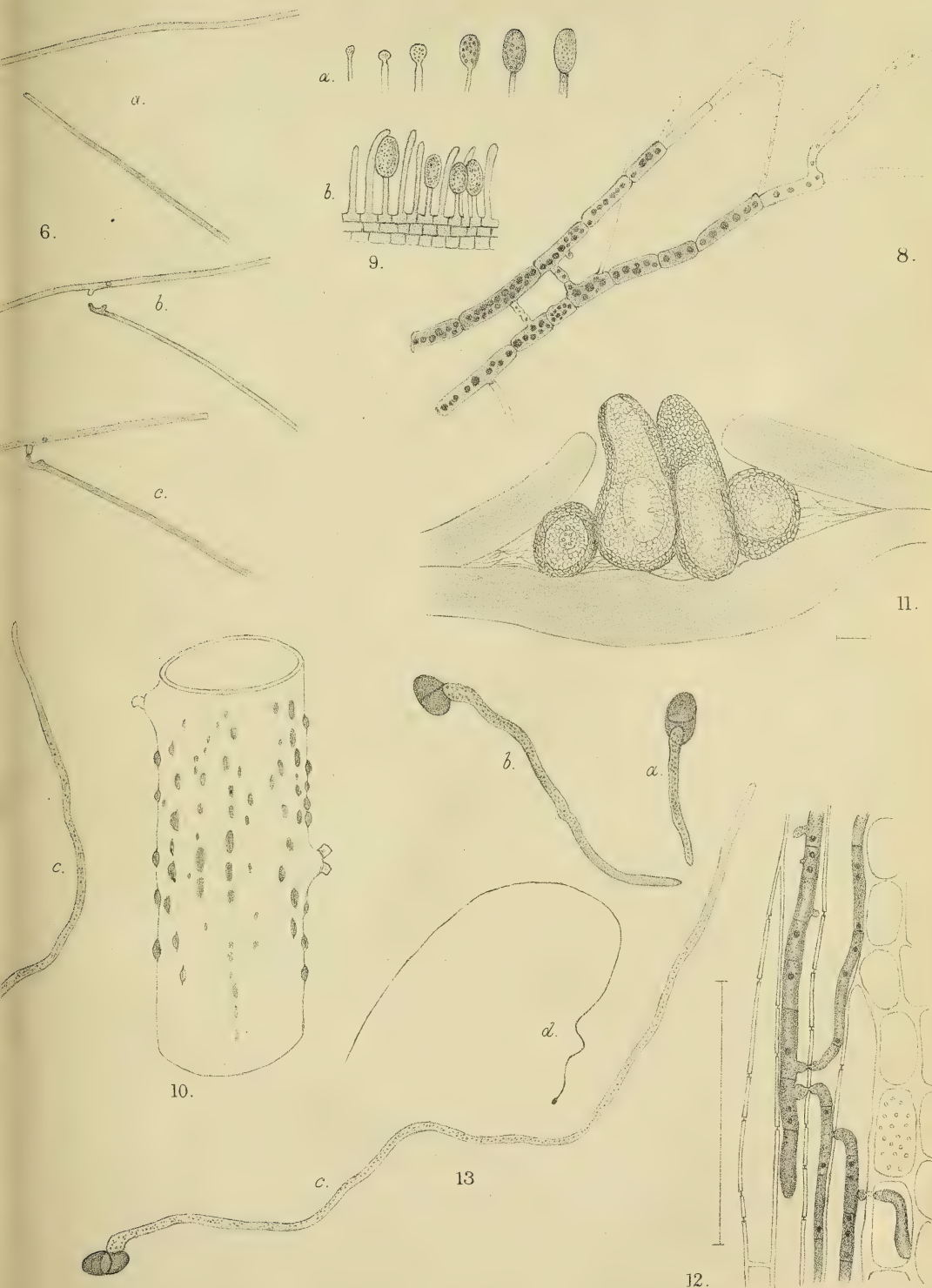
Fig. 10. A portion of a cacao branch attacked by the Fungus (which has been kept in a moist chamber for a short time), showing the pycnidial colonies breaking through the bark. Nat. size.

Fig. 11. Transverse section through a pycnidial colony bursting through the bark of a cacao branch. The second pycnidium on the left hand shows the ostiole. Zeiss. *aa*.

Fig. 12. Portion of a tangential section of a cacao branch, showing the mycelium passing through pits in the walls of the vessels. A portion of a medullary ray is shown on the right-hand side. The mycelium is brown, and shows numerous oil-drops. Zeiss. *E*.

Fig. 13. Stages in the germination of a spore obtained from a pycnidium on a diseased cacao branch. The sowing was made at 2 p.m., Dec. 18: *a*, at 4.5 p.m.; *b*, at 4.45 p.m.; *c*, at 6.45 p.m.; and *d*, at 11 p.m. the same day. Temp. 27-29°C. At 7.0 a.m., Dec. 19, there was a copious development of a branched, rarely septate mycelium in the drop, which was far too complicated for me to draw. *a, b, c* $\times 375$; *d* $\times 60$.







HOWARD. — ON DIPLODIA.

Comparative Anatomy of the Hymenophyllaceae, Schizaeaceae and Gleicheniaceae.

III. On the Anatomy of the Gleicheniaceae¹.

BY

L. A. BOODLE, F.L.S.



With Plates XXXVIII and XXXIX.



THE fullest account of the anatomy of the Gleicheniaceae previously published is that given by Poirault ('93), but although he enters into a careful description of the petiole and node in different species of *Gleichenia*, the structural characters of the Order have in some respects been less completely dealt with than was the case with the Hymenophyllaceae and Schizaeaceae, which formed the subject of two monographs by Prantl ('75 and '81).

The range of diversity in the structure of the rhizome is less in the Gleicheniaceae than in the Schizaeaceae. In the latter Order four main types of stelar structure were recognized, while in the Gleicheniaceae only three are found, as will be described below. No case of dialystely has so far been discovered. The homologies of the three types of structure which occur in the Gleicheniaceae is an interesting question, but, although it is as well to discuss it, there does not

¹ From the Jodrell Laboratory, Royal Botanic Gardens, Kew.

appear to be any immediate prospect of a definite solution to such morphological problems.

Two genera only are included in the Gleicheniaceae: *Platyzoma*, Br., and *Gleichenia*, Sm.; the species, on which *Stromatopteris*, Mett., was founded, being classed by Hooker and Baker ('74) as a species of *Gleichenia*. By both Christ ('97) and Diels ('00) *Platyzoma* is included in *Gleichenia*, and according to the latter author *Stromatopteris* ranks as a genus distinct from *Gleichenia*.

It is hoped to obtain further material of some of the species in which only small pieces of dried material were available, and to publish some additional observations together with those proposed in the case of the Schizaeaceae.

Of the two genera, *Gleichenia* will be taken first, and the description will be divided into sections in the same way as in the last part of this paper (p. 359 of this volume).

Below is a list of the species in which either rhizome, or petiole, or both were examined. This list is given here so that the specific names may be divested of their authors in other parts of the paper.

Platyzoma microphyllum, Br. In the subgenus *Eugleichenia*, *G. moniliformis*, Moore, *G. polypodioides*, Sm., *G. circinata*, Sw., *G. Boryi*, Kze., *G. dicarpa*, Br. In the subgenus *Mertensia*, *G. longissima*, Bl., *G. flabellata*, Br., *G. Cunninghami*, Hew., *G. pedalis*, Klfs., *G. cryptocarpa*, Hk., *G. revoluta*, H.B.K., *G. simplex*, Hk., *G. pubescens*, H.B.K., *G. flagellaris*, Spr., *G. vestita*, Bl., *G. pectinata*, Pr., *G. dichotoma*, Willd.

GLEICHENIA, habit.

The stem, except in *G. moniliformis*, is a creeping rhizome, bearing roots on its lower side and leaves on its upper side. The internodes are usually long, and the leaves, though sometimes apparently forming a single row, are in certain species tristichous, though with only a small divergence. The roots may be irregularly placed, or in rows (e. g. tristichous).

The leaf generally has a long petiole, and the first division is dichotomous, dividing the leaf into two primary pinnae,

but with a bud at the fork, which may continue to grow for a time as a main rachis, and then repeat the process of forking and production of a terminal bud. Thus many pairs of primary pinnae are formed, and their mode of growth may be identical with that of the main rachis. The pinnules are borne in a pinnate manner. Diagrams illustrating the leaf-form in the different sections of the subgenus *Mertensia* are given by Diels ('00, p. 353). The dichotomy of the primary pinnae, and the production (at the fork) of a bud, which afterwards develops as a secondary rachis, and the pinnate arrangement of the pinnules, are all characters which are also found in species of *Lygodium*. Further, in *G. pectinata* after the first dichotomy the rachis, which is perhaps a sympodium, bears pinnae singly, and as the latter are short and forked there is a close agreement with what is found in *Lygodium*. *G. moniliformis* and *G. simplex*, both of which appear to be reduced forms, have simple pinnatipartite leaves. Excluding these, dichotomy in the leaf is a character of the genus. It is a primitive character which is shared by species of *Lygodium*, *Schizaea* and *Gleichenia*. It may be pointed out as quite possible that the pinnate form of leaf, as found in *Anemia* and perhaps in some other groups of Ferns, may have originally been derived from a dichotomous form of leaf, like that of *Gleichenia*, the pairs of primary pinnae representing dichotomies, and the rachis representing the products of a series of buds formed in the successive forks. In other cases a pinnate leaf may occur in a dichotomous type, apparently by suppression of dichotomy, what would be the ultimate branches of the leaf being borne directly on the petiole.

Numerous forms of hairs and paleae occur on the rhizome and leaf.

GLEICHENIA, rhizome.

In the majority of the species of *Gleichenia* the rhizome has a uniform type of structure in the internode, namely, a centrally placed solid stele (protostele), consisting of a central mass of xylem, composed of tracheides and paren-

chyma, and surrounded by a continuous ring of phloem, pericycle and endodermis. There is thus a general resemblance to the structure of the rhizome of *Lygodium*, but a point of difference is that in *Gleichenia* the xylem is mesarch, and has several distinct groups of spiral protoxylem-elements.

The only other widely different type of structure found in the genus is the solenostelic, which has so far been observed only in *G. pectinata*. It will be convenient to describe the species, which possess a protostele, first, and to treat *G. pectinata* separately.

In each species the diameter of the rhizome is of course liable to variation according to the vigour of the specimen, but two examples will give an idea of the sizes met with. A rhizome of *G. polypodioides* measured about 1 mm. in diameter, while one of *G. pubescens* reached rather more than 3 mm. These, as compared with other species, may be taken as a small and a large rhizome respectively. The proportionate dimensions of stele and cortex in *G. flabellata* are shown in Fig. 1, Plate XXXVIII. In this diagram *a.* represents the epidermis and outer 2-3 layers of the cortex, whose cells have colourless and comparatively thin walls; *b.* and *c.* are the middle and inner zones respectively of the cortex, and consist of brown-walled sclerotic tissue, which is very thick-walled in proportion to the size of the lumen in *b.*, and less so in *c.* There is no very sharp line of demarcation between *b.* and *c.*, so the limit taken in the diagram is where the sclerenchyma begins to be appreciably thinner walled.

In most species the cortex consists chiefly of brown sclerotic tissue, of the kind so frequently found in the cortex of other Ferns. Thus the sclerotic tissue of the cortex of *G. dichotoma* is composed of long brown-walled fibrous elements with oblique end-walls, but divided into shorter elements by transverse septa. The walls of the fibres bear small oval pits. The length of the fibrous elements is however variable, as one piece of rhizome of the same species was found to have quite short sclerotic cells in place of the long fibres.

Several species resemble *G. flabellata* in having the epidermis

and the succeeding 1-2 or 3 layers of the cortex comparatively thin-walled, e.g. *G. vestita*. In *G. dichotoma* the epidermis and the next 1-2 layers contain mucilage, and are either sclerotic and brown-walled, or, especially on the lower side of the rhizome, they may have colourless and not very thick walls. In *G. pedalis* the epidermis and the whole of the cortex are brown-walled, the walls being of nearly uniform thickness throughout these tissues.

In the cortex of the rhizome in most species small intercellular spaces are found at the corners of the sclerotic cells; these spaces are, however, usually partly or nearly completely filled with a yellow, granular substance. It was not determined whether the intercellular space was first formed, by the splitting of the walls, and then partly filled by a granular deposit, or whether the space and the granules were produced simultaneously by the absorption of some of the constituents of the middle-lamella. The granular matter may perhaps consist of pectic substances, similar to the rodlets found between the parenchymatous cells of the petioles &c. of several Ferns (see Poirault, '93, p. 243), but infiltrated with the brown colouring matter (phlobaphene) found in the sclerotic membranes. In the rhizome of *G. moniliformis* intercellular spaces between the sclerotic cells appear to be absent.

Surrounding the stele there is a well-marked endodermis, which shows the usual characters in its radial walls. Its cells, in transverse section, often have a tangential diameter one-and-a-half times to twice the radial diameter (Figs. 3 and 6, e). The radial walls may become yellow in the mature rhizome. The endodermal cells usually abut directly on the sclerotic cortex (Fig. 3), but occasionally the outer tangential walls of the endodermis remain thin. In *G. dicarpa* and *G. circinata* the endodermal cells contain mucilage, and in *G. flabellata* they contain a colourless finely granular body, which is probably siliceous.

The pericycle is 2-5, generally 3-4 cells thick, its thickness varying according to the size of the stele. Its cells are

usually of about the same tangential diameter as the endodermal cells, but are more nearly isodiametric. In the mature stem they do not generally show definite radial seriation, but in a transverse section of the young rhizome of *G. dicarpa* the pericyclic cells at many points were neatly arranged in radial rows, which did not come to an end with the pericycle but were continued through the endodermis to the next two or three layers of the cortex. At other points the radial rows were disturbed by displacement of cell-walls, but this was so slight that their radial origin was still fairly clear. The pericycle therefore appears to be cortical in origin.

The protophloem forms a practically unbroken layer of small elements (sieve-tubes and phloem-parenchyma) similar to those in *Lygodium*, and taking a strong stain when treated with Kleinenberg's haematoxyline. The layer of protophloem may be recognized in the low-power photograph (Fig. 1, *pph.*), and is shown as it appears under the high-power in Fig. 3 (*pph.*). The metaphloem forms a continuous zone within the protophloem, and consists of large sieve-tubes and a certain amount of parenchyma. The walls of these sieve-tubes are seen to be of considerable thickness when the section is mounted in glycerine-jelly or in weak glycerine (*s.t.*, Fig. 3). Numerous refractive granules are often seen adhering to the walls as in other Ferns. As the sieve-tubes were not found to differ in any important point from those of *Lygodium*, as to the character of their sieve-plates, &c., there is no need to enter into detail, except as to the presence or absence of callus. Sections of the rhizome and petiole of two or three species of *Gleichenia* were tested with azoblue, but gave no evidence of the presence of callus. Subsequently the rhizome of *G. pectinata* was examined. In this case dried material was used, and a section was cut, and tested with azoblue, soon after the material had been softened by boiling in water. It was then found that a few of the sieve-tubes showed distinct callus-knobs on their sieve-plates, similar to those which occur in *Pteris aquilina* (Boodle, '01, p. 399). An

attempt to obtain similar preparations, after the material had been left in spirit for about two days, entirely failed. This indicates the possibility that in the other species of *Gleichenia* and in the Hymenophyllaceae and Schizaeaceae, where the azoblue-test failed, the callus-reaction might be obtained by means of some preliminary treatment, e. g. boiling for a short time; though this is unnecessary in the case of *Pteris* and many other Ferns. That is, either callus may be present, but masked by being infiltrated with some other substance, which prevents the appearance of the characteristic stain, or there may here be a body slightly differing from callus, but easily converted into it. Poirault ('93, p. 191), also working with dried material, mentions having found callus in the sieve-tubes of the *Gleicheniaceae*. Unstained preparations from dried material in two or three species showed bodies strongly resembling callus-rods and knobs.

A layer of parenchyma separates the metaphloem from the xylem (see Fig. 3). The xylem forms the central part of the stele. Its diameter varies roughly with that of the rhizome. In *G. polypodioides* it measures only about $\frac{1}{3}$ mm., while in *G. pubescens* it reaches $1\frac{1}{3}$ mm. In several species the xylem has an approximately circular outline in the internode (Fig. 1, *x*), but becomes oval near the node (Fig. 2). *G. flabellata* and *G. pubescens* are two of the species, which have a circular xylem. In others it is distinctly lobed, and the lobes have a definite relation to the protoxylem-groups, e. g. in *G. dichotoma* (Fig. 14, *x*), *G. dicarpa*, *G. circinata*. When the xylem is lobed the metaphloem forms a thicker layer, and is composed of larger sieve-tubes in the bays of the xylem than opposite the lobes. The structure of the xylem can be seen by comparing Figs. 2, 3, 4 and 5. The general low-power view of the xylem in Fig. 2 (*G. flabellata*) shows that the tracheides are arranged in chains and groups, separated by parenchyma as in *Lygodium*. The essential point of difference lies in the position and nature of the protoxylem. In *Gleichenia* there are several small groups of annular and spiral protoxylem-tracheides placed a short

distance below the periphery of the xylem, the remainder of the tracheides being scalariform. In Fig. 2 about fourteen protoxylems are present; some of these may be recognized as groups of small darkly stained elements (*px.*). Fig. 3 contains a peripheral piece of the xylem of *G. dichotoma*, showing the scalariform tracheides separated by xylem-parenchyma (*x.p.*). Fig. 4 represents one of the lobes of the xylem of *G. circinata*, and shows the mesarch position of the protoxylem (*px.*). Part of a young stele of *G. dichotoma* in Fig. 6 is at the stage in which the protoxylem (*px.*) is the only part of the xylem differentiated; young tracheides (*t., t.*), which have not yet begun to thicken their walls, are seen on the outer side of the protoxylem. Fig. 5 is a radial section of the outer part of the stele of *G. circinata* showing the protoxylem (*px.*) with one scalariform tracheide on the outer side; *pph.* is a protophloem element. Fig. 14 may be referred to for the relation of the protoxylem-groups to the lobes of the xylem in *G. dichotoma*. In species where the xylem is lobed, there is nearly always a protoxylem immersed in or below the xylem of each lobe, though there may sometimes be additional protoxylem-groups having no corresponding prominences.

In the internode of *G. dichotoma* the xylem generally shows six prominences with six protoxylems. The lobes are fairly symmetrically placed, so that the xylem may be described as roughly hexagonal, with rounded angles (Fig. 14). The three lobes on the lower half of the xylem serve for the attachment of roots, though a root is occasionally inserted on one of the upper lateral lobes, while the three protoxylems in the upper half are connected with leaf-traces. A rhizome of *G. dichotoma*, var. *nervosa*, which was larger than that of the type, had a xylem with 8-9 lobes. In *G. circinata* there are often six as in *G. dichotoma*.

In these species the xylem usually has a nearly symmetrical form, with protoxylem-groups equal in number to the lobes. In *G. dicarpa*, on the other hand, there are often 7-8 protoxylems, of which 2-3 on the upper side have no distinct

xylem-prominences connected with them, the outline of the xylem being rounded or nearly flat on this side, while the remainder of the protoxylems are found below well-marked lobes. This structure was seen, for instance, at a distance of about $\frac{3}{4}$ inch from a node.

A similar rounding or flattening of the upper side of the xylem occurs as one approaches the node in *G. dichotoma*, but in *G. dicarpa* and some other species it is found through most or the whole of the internode, so that the xylem only bears prominences below and at the sides.

G. dicarpa, var. *alpina*, has a much smaller rhizome than the type, and a rounded-quadrangular xylem, with a protoxylem embedded in each angle.

Once more comparing the structure of the rhizome of *Gleichenia* with that of *Lygodium*, and leaving the striking differences in the protoxylem out of the question, a few points of difference in detail may be mentioned. In *Gleichenia* there is usually a greater proportion of parenchyma in the xylem, and it more often occurs as 2-3-seriate chains of cells (instead of uniseriate); the tracheides are often broken up into shorter chains or groups, are often larger in proportion to the size of the stele, and frequently have a shorter common wall, where they are in contact with one another. Owing to the latter point and to the tracheides being in contact with a larger number of parenchyma-cells, the tracheides of *Gleichenia* in longitudinal section less frequently show the scalariform pits running right across the width of the elements than is the case in *Lygodium*. All the above small differences are probably liable to exception.

Having given a general description of the structure of the rhizome of *Gleichenia*, some peculiarities of certain protostelic species should be mentioned. In a section of the rhizome of *G. dicarpa* a few of the xylem-parenchyma-cells were seen to be thick-walled and lignified, and others showed traces of lignification at their corners in the middle-lamella, so it is quite likely that a general lignification of these cells may take place in quite old parts of the rhizome. Poirault ('93, p. 173)

mentions the rare occurrence of sclerotic cells among the scalariform tracheides in the rhizome of this species.

In *G. cryptocarpa* mucilage occurs in the xylem- and phloem-parenchyma, and in *G. dicarpa* it is found in the same cells as well as in the pericycle.

G. moniliformis is the most aberrant species in that no protoxylem-elements are distinguishable in the mature stem, and the tracheides all appear to be scalariform. The stele is small, the xylem measuring rather less than $\frac{1}{8}$ mm. in diameter. The absence of spiral protoxylem-elements is interesting, because this species is the only one of its genus with an upright stem. It was noted among the Hymenophyllaceae, where spiral protoxylems are general, that their absence in certain species of *Trichomanes* appeared to be correlated with an upright habit (*T. spicatum* and *T. Bancroftii*). Both *G. moniliformis* and the species of *Trichomanes* just mentioned have probably been derived from forms with a creeping rhizome, and the change to the upright stem has been accompanied with diminished length of the internodes and slower growth of the rhizome. This makes it easy to understand the disappearance of the spiral elements.

In the rhizome of *G. Cunninghami* the xylem in transverse section presented a curious appearance on account of the presence of a rather neat uniseriate peripheral band of large tracheides, which was continued about half way round the circumference, with only four of five inconspicuous interruptions by parenchymatous cells. This was probably a band of tracheides ready to form the chief part of a leaf-trace, though at some distance from a node.

The depth of the protoxylem-groups below the periphery of the xylem differs in the various species. *G. vestita* may be mentioned as a case in which they are deeply seated in proportion to the size of the xylem.

Lignified sieve-tubes were observed by Poirault ('93, p. 195) in the stem of *G. polypodioides*. Similar elements were not seen by the writer in the stem of this or any other species

of *Gleichenia*, but this may be due to the rhizomes examined not being sufficiently old.

When the rhizome branches, the stele divides in a simple manner as in *Lygodium*, but, in the cases observed, one stele is smaller than the other and passes off laterally. Both this character and the external appearance suggest monopodial branching, not dichotomy.

GLEICHENIA, petiole.

In most species of *Gleichenia* the cortex of the petiole bears a general resemblance to that of the stem. It consists largely of the usual brown-walled sclerotic tissue, which may be fairly uniform throughout, or may fall into two zones, one of which is formed of much thicker-walled elements than the other, or into three zones of which the middle is the thinnest-walled. The epidermis and sometimes one or two adjacent layers of the cortex may be comparatively thin-walled, as occurs in some rhizomes, or may be strongly sclerotic.

The structure of the petiole has been described by Poirault ('93, p. 176, &c.), and two detailed drawings (Figs. 17 and 18) are given by him of the petiolar bundles of *G. Speluncae*, Br., and *G. rupestris*, Br. Both of these belong to *Eugleichenia*. *G. Speluncae* is given by Hooker and Baker ('74, p. 11) as a synonym of *G. circinata*, and *G. rupestris* as a separate but very nearly allied species. *G. rupestris* has, however, been since reduced by Baker ('91, p. 183) to a variety of *G. circinata*.

The type of structure in the petiole is described by Poirault ('93, p. 189) as being very different in the two subgenera of *Gleichenia*, and the difference is illustrated by his diagrams of the separated leaf-traces (Figs. 13 *c* and 14 *d* for *Eugleichenia* and 16 *d* for *Mertensia*). The distinguishing point is that the bundle is circular or subcordiform in *Eugleichenia*, but more or less shaped like a C in *Mertensia*. This distinction appears to hold good for the majority of species; however in *G. (Mertensia) pedalis* the indentation of the bundle is only slight, so that its form is about reniform, while

in *G. (Mertensia) dichotoma* the form of the bundle is as in *Eugleichenia*. In the latter species the internal structure of the bundle might be regarded as giving evidence of derivation from the usual type for *Mertensia*.

G. dicarpa may be taken to illustrate the type of petiolar structure found in *Eugleichenia*. Fig. 7 is a transverse section of the bundle. The xylem has the form of an arch with incurved ends, and possesses three protoxylem-groups. The position of the latter is indicated by the three cells marked *c.p.* in the drawing, which are elements resembling cavity-parenchyma. There is a protoxylem-group consisting of annular and spiral tracheides (some of which are often crushed) in contact with the outer side of each of these cells, i. e. radially external to them. It is thus seen that the xylem agrees with that of *Anemia Phyllitidis*, in that three protoxylems are present, and are internal to the xylem-arch, one being median, and the other two in the concavity of the hooked ends of the xylem. The phloem in *G. dicarpa* clothes the external surface of the xylem-arch, and bends inwards round its ends, following its outline, and continuing upwards towards the top of the arch, e.g. about three-quarters of the way. The phloem has the same constituent elements as in the stem, but has fibrous elements in addition. The proto-phloem, forming the boundary of the phloem towards the pericycle, can be recognized by its small elements (*pph'*). The latter are fairly thick-walled, but have not been represented so in the drawing, in order that the phloem-fibres might be distinguished by this character. The phloem-fibres are seen at *f.*, *f.*, and at the two corresponding points in the other half of the bundle. They have been formed by thickening and lignification of the walls of phloem-elements; both sieve-tubes as described by Poirault ('93, p. 195) for *G. circinata* and *G. dicarpa*, and also phloem-parenchyma, being thus transformed. A few similar fibres may occur in the phloem outside the top of the xylem-arch. The central region of the bundle probably represents pericycle, and consists partly of parenchyma and partly of sclerotic

elements (*sc.*), which have not the brown walls characteristic of the cortex. The pericycle on the outer side of the bundle is 3-4 cells thick, and bounds the drawing. In a section of another petiole of *G. dicarpa* each of the two lateral protoxylems was split into two slightly separated groups. The difference in structure at the base of the petiole in this species will be described in connexion with the node.

The other species of *Eugleichenia* have petioles of the same type as *G. dicarpa*; that is, the endodermis is not invaginated, the xylem forms an arch, and three similarly placed protoxylem-groups are present.

There are considerable differences in the size and proportion of parts in these other species, but the type of structure is practically the same. In *G. circinata* the structure is very similar to that of *G. dicarpa*. In *G. Boryi*, which has a much smaller petiole, no sclerotic cells are differentiated in the central tissue, and fibrous constituents of the phloem appear to be quite absent. In *G. moniliformis* also the bundle is small, the xylem-arch has more the form of a narrow U, in which the hooks are hardly represented, the phloem is only continued a short way up in the interior, and only about two sclerotic elements were found in the central parenchyma. In *G. polypodioides* the structure at first sight looks very different, as it shows an almost solid-looking mass of xylem, shaped something like an equilateral triangle with rounded angles. It may, however, be referred to the same type as the other species. The differences of detail are as follows:—in *G. polypodioides* the central mass of tissue (which in *G. dicarpa* includes the sclerotic tissue, *sc.*) is only represented by a narrow line of parenchyma, one to two cells thick¹; the hooks of the xylem have only narrow bays, containing parenchyma and fibrous phloem-elements, which are continuous with the phloem bending in through the small space

¹ In *G. circinata* a transverse section of a young petiole, with protophloem and protoxylems differentiated, showed an arrangement of the cells in the central mass of parenchyma, which gave evidence of comparatively late cell-division, apparently deriving the group from a single median row of cells.

between the tips of the hooks. The protoxylem groups have the usual positions. A few sclerotic elements are found in the phloem on the outer side of the xylem, chiefly opposite the lateral protoxylems.

Poirault ('93, p. 190) mentions as a distinguishing character, that all the species of *Eugleichenia* except *G. Boryi* possess strongly lignified, pitted or reticulate cells in the pericycle of the petiole, while in *Mertensia* such cells occur only isolated, or in the majority of species are completely absent. This cannot be held as a reliable distinction. Among species of *Eugleichenia*, *G. dicarpa* showed lignification and slight thickening of its pericyclic cells, and *G. dicarpa*, var. *alpina*, had a pericycle composed entirely of pitted and strongly sclerotic cells¹. *G. dicarpa*, var. *longipinnata*, also has a sclerotic pericycle. On the other hand, sclerotic pericyclic cells were not observed in *G. circinata*, though they were found by Poirault in *G. rupestris*, which is to be regarded as a variety of this species. Turning to *Mertensia*, *G. cryptocarpa* has its pericycle sclerotic throughout as in *G. dicarpa*, var. *alpina*, though not quite so thick-walled. As *G. cryptocarpa* and *G. dicarpa*, var. *alpina*, are both distinctly xerophytic, it appears probable that xerophytic habit may produce a strongly sclerotic pericycle in both subgenera, though *Eugleichenia* may have a more general tendency to form it than *Mertensia*.

We may now deal with the petiole of *Mertensia*. Some of the species have much larger petioles and petiolar bundles than are found in the other subgenus. The type of structure of the bundle may be said to be much the same as in *Eugleichenia*, with the exception that the endodermis is invaginated, so as to follow to some extent the outline of the xylem-arch, and so that the central region is occupied by sclerotic tissue continuous with the cortex, and apparently belonging to it, instead of to the pericycle as in *G. dicarpa*. Thus the bundle is arched, or in several species roughly C-

¹ Some of the more external of these had their walls slightly tinged with the brown colouring matter, which usually characterizes the cortical sclerenchyma.

shaped, as described by Poirault. The points of agreement with the *Eugleichenia*-type are best seen in the species of smaller stature, e. g. in *G. simplex*, where the petiolar bundle is rather smaller than that of *G. circinata*. The xylem here has the form of an arch with sharply hooked ends, has a median and two lateral protoxylems in the usual positions; the phloem is continued on the inner side of the xylem to about the same level as in *G. dicarpa*, and its elements are fibrous in the concavities of the xylem-hooks.

A point of difference from the other subgenus is seen in the incurving of the endodermis, which may be described as having been pushed into the arched bundle by a promontory of cortical sclerenchyma. Other points of difference from *Eugleichenia*, which would apply to a fair proportion of the species, are that in *Mertensia* the xylem-arch is composed of a continuous zone of tracheides of fairly uniform size, not interrupted by considerably smaller tracheides opposite the protoxylem-groups, and secondly that there is often a conspicuous group of small tracheides between the median protoxylem and the large tracheides forming the top of the xylem-arch.

In species of *Mertensia*, where the petiolar bundle is larger than in *G. simplex*, the protoxylem-groups become more and more numerous according to the size of the xylem-arch. Thus both the median and the two lateral protoxylems may each be represented by two groups, making six in all, or, to take an extreme case, in a large form of *G. longissima* about twenty groups were counted. The leaf-trace in the upper part of Fig. 27 shows the form of petiolar bundle found in some of the larger *Mertensias*.

The structure of the petiole of *G. dichotoma* differs from that of other species of *Mertensia* in that the bundle is bounded externally by an endodermis, which is not incurved, but in the central tissue of the bundle is found a mass of sclerotic tissue surrounded by a complete ring of endodermis¹

¹ Something similar to this is found in the basal part of the petiole of some *Eugleichenias*, to be described later, in dealing with the node.

(Fig. 8), or this mass may be broken up into three (Fig. 9). The difference between *G. dichotoma* and the *Eugleichenia*-type can be seen by comparing Figs. 9 and 7. Some of the phloem in the hooks of the xylem is converted into fibrous elements in *G. dichotoma* just as in *G. dicarpa*, and similar cavity-parenchyma is found in connexion with the protoxylem-groups.

Cells which are evidently of the same nature as the cavity-parenchyma mentioned in the Schizaeaceae (Boodle, '01, p. 394) are found adjoining the protoxylems in the petiole of nearly all *Gleichenias*. They are cells which have evidently undergone extension towards the protoxylem-elements, either as the cause or effect of the crushing of the spiral tracheides, but in *Gleichenia* they generally have flattish walls, as seen in longitudinal section, and so do not present the character on which the name 'Lückenparenchym' was based. Fig. 10 shows three cells of this tissue (*c.p.*) in the petiole of *G. dichotoma*. One of them has undergone late division; and crushed protoxylem-elements are seen at *px'*. Throughout the genus it frequently occurs that a considerable number of the cavity-parenchyma-cells become rather thick-walled, pitted and lignified.

A small part of the petiolar bundle of *G. dichotoma* is shown as a high-power drawing in Fig. 11, where *sc.* is the left-hand end of the central sclerotic mass of Fig. 8. The sclerotic group is surrounded by an endodermis mostly torn (*i.e.*); *px'* is part of a crushed protoxylem-group; *ph'* and *pph'* are the meta- and protophloem of the incurved phloem, and the terminal part of it is replaced by fibres *f*. Two of these probably represent sieve-tubes, and the other two phloem-parenchyma. The sclerotic elements in Fig. 12 are some from the central mass in *G. dichotoma*, shown in longitudinal section. Many of the elements of this tissue are much longer, more pointed and thicker-walled (*i.e.* more fibrous) than those in the figure.

Before leaving the petiole, one or two peculiarities of structure in certain species of *Gleichenia* deserve notice. In *G. circinata* a granular body, apparently siliceous, was

observed in most of the endodermal cells of the petiole, both in the type and in the form originally separated as *G. rupestris*. These bodies, which are just like those in the endodermis of the stem of *G. flabellata*, were not seen in the petiole of any other species besides that mentioned.

In the bundle of *G. pedalis* the endodermis is only slightly incurved, so there is room for a mass of sclerotic pericycle, which is present here as in some *Eugleichenias*, between the endodermis and the cavity-parenchyma accompanying the median protoxylem. The cavity-parenchyma, as in several other *Mertensias*, forms a conspicuous group of lignified cells.

In the large form of *G. longissima* previously mentioned the fibres of the bundle are specially numerous. The bundle is of large size, and the fibres extend practically all the way round the inner face of the xylem. Opposite the terminal protoxylems they form a nearly continuous layer, and appear to belong to the phloem, elsewhere they are more scattered and perhaps represent pericycle.

The petiole of *G. cryptocarpa* is remarkable, as it contains three bundles instead of one. They are not widely separated, and if pushed into contact would form a typical C-shaped bundle with the usual orientation. There is one long slightly curved bundle, and two shorter sharply curved ones, representing the long side and the two hooked ends of the C respectively. As a single leaf-trace passes off from the stele to supply the leaf, and is continued into the base of the petiole as an arched bundle of the usual form for *Mertensia*, the peculiar structure described above is to be explained as due to a very early separation of the bundles for the first two pinnae¹. This division of the bundle generally takes place quite a short distance below the pinnae, but here it had already occurred at fourteen inches below the first pinnae.

In *G. flagellaris* and some other species each incurved end of the xylem-arch does not terminate in a hook, but in a knob, in the middle of which a protoxylem-group is placed. This feature may be more or less restricted to the upper part of

¹ The same thing appears to take place in *G. flagellaris* (Poirault, '93, p. 176).

the petiole, and may be described as being due to the folding back of the hook until the bay of parenchyma disappears. This may be illustrated in *G. dichotoma* by comparing Figs. 9 and 8.

GLEICHENIA, node.

Diagrams of series of sections through the node, illustrating the mode of separation of the leaf-trace, have been given by Poirault ('93, p. 172 et seq.) for two species of *Eugleichenia* and one of *Mertensia*. Poirault's description of these makes most of the features clear, but as the nodal structure is very interesting, and as one or two particulars appear to have escaped Poirault, two series of diagrams are given here (Figs. 14-25), viz. one series for *Mertensia* and one for *Eugleichenia*. In examining the separation of the leaf-trace in three or four nodes of *G. dichotoma*, it was found that several differences of detail occurred. Probably the same would be found to apply to other species also.

Fig. 14 represents the internodal structure of *G. (Mertensia) dichotoma*. Here we may speak of three upper and three lower protoxylem groups, or of two median and four lateral. Figs. 15-19 are diagrams of a series of sections through one particular node, which will now be described. On approaching this node¹, the upper median protoxylem travels towards, and then fuses with, one of the adjacent protoxylems, thus reducing the number of protoxylem-groups to five, which are placed as seen in Fig. 15. The protoxylem-group, A, is the one that has been formed by the fusion of two. In succeeding sections the protoxylem, B, multiplies its constituent tracheides, and then forks into two, the parenchyma adjacent to it having meanwhile increased in amount, so as to produce an island in the xylem, which may be called the 'nodal island,' as seen in Fig. 16, where *n.i.* is the nodal island, and *b., b.* are the protoxylems produced by the splitting of B. In the next stage (Fig. 17) the upper of these has travelled

¹ This and other nodes described are explained by beginning below and going in the acropetal direction.

upwards to b ., while the lower one has divided into b^1 . and b^2 ., and A has divided into a ., a . The lower a . and also b^2 . will remain in the stele, while the upper a ., b . and b^1 . will pass out as the three protoxylems of the leaf-trace. In the stage we are considering (Fig. 17) the nodal island (*n.i.*) has increased greatly in size, and other tissues besides parenchyma have become differentiated in it. There is a central mass of brown sclerotic tissue (*sc.*) surrounded by an endodermis, and on the lower side of the island are a few sieve-tubes, represented by short lines in the diagram. Detailed drawings of a similar nodal island are given by Poirault ('93, p. 175, Fig. 15) for *G. pubescens*, H. B. K., var. (under the syn. *G. furcata*). As described by Poirault, the first appearance of the sclerotic group, as one goes towards the node, is one fibre sheathed by endodermis; then more and more fibres are added until the group, still surrounded by an endodermis, attains considerable size. Passing on from Fig. 17, the thickness of the parenchyma increases on the inner side of the sclerotic group, the phloem in the nodal island increases in amount, and splits into two bands, which become separated by a bar or bridge of xylem appearing between them, as seen in Fig. 18, Pl. XXXIX. In the next stage (Fig. 19), the xylem of the leaf-trace has become separated from that of the stele by the splitting of this bar of xylem. Consequently part of the original nodal island passes off inside the leaf-trace, and contains the sclerotic tissue and a band of phloem; while part of the nodal island remains enclosed in the xylem of the stele (*n.i.*). It still contains a few sieve-tubes, but these soon disappear, and the parenchymatous island shrinks in size, until it is reduced to a few cells accompanying the protoxylem b^2 . in the internode. A new upper median protoxylem is supplied by the division of a ., and the typical internodal structure is restored.

In a second node of *G. dichotoma*, the xylem of the leaf-trace again passes off closed; the only difference of detail is that, in the stage a little later than Fig. 17, three or four sclerotic elements, apparently surrounded by an endodermis, branch away from the main mass at the same time that the

phloem-band splits, and pass into the stelar part of the nodal island, but die out upwards almost at once, i. e. at the level of the first appearance of the xylem-bridge.

In a third node, when the leaf-trace separates, its xylem is closed, as in the previous cases, but becomes open while the leaf-trace is still in the cortex of the stem.

In the fourth node examined, the bar of xylem which usually appears in the nodal island, cutting it into two, is not formed, and the leaf-trace consequently passes off from the stele with an open xylem-arch, leaving the inner part of the original nodal island as simply a bay in the exterior of the xylem. In this case the phloem in the nodal island and the internal phloem of the leaf-trace both have a connexion with the external phloem of the stele, though this was of course not the case in the other three nodes.

In the last two nodes mentioned, the leaf-trace passes off in the median plane of the stele, i. e. from a point on the opposite side from the lower median lobe, not obliquely as in Fig. 18. It is no doubt in connexion with this that the behaviour of the protoxylems is much simpler than in the first node described. In the last two nodes, the upper median protoxylem of the internode passes out as the median protoxylem of the leaf-trace, whose two lateral protoxylem-groups are supplied by forking of the two upper lateral protoxylems of the internode. Thus the behaviour of the protoxylems and the position of the leaf-trace as it leaves the stele show that the leaf-arrangement should be regarded as tristichous, median and slightly lateral leaf-traces being produced.

The petiole, belonging to the second node described, was $14\frac{1}{2}$ cm. in length, and was sectioned at close intervals. The results need not be given in detail, but one or two changes in structure should be referred to. The central sclerotic mass with its investment of endodermis divides into three, and then fuses again (as in Figs. 9 and 8). It appears to remain permanently enclosed in the bundle, having no connexion with the cortex or the outer endodermis. The xylem of the bundle in this petiole remains closed until a height of 4-5 cm. is

reached, when it opens, and allows continuity first between the inner and outer phloem (Fig. 9), and then between inner and outer pericycle (as in Fig. 8). Near the top of the petiole the reverse changes are gone through, so that the xylem becomes closed again just before the separation of the pinnae.

G. dichotoma has been described at length, because its petiole being of an exceptional type, the nodal structures differ in several points from Poirault's description of the node of *Mertensia*, and because the completely enclosed sclerotic tissue with its endodermis is an interesting feature.

The node of *G. flabellata* may probably be taken as fairly typical for *Mertensia*, and agrees in most points with the diagrams given by Poirault ('93, Fig. 16, p. 177) for that subgenus. The structure is as follows:—A nodal island appears in the stele, a few sieve-tubes appear near the middle of the island (*i. ph.* in Fig. 2); the sieve-tubes increase in number, and shift their position, so as to lie near the periphery of the island, forming an arc on the inner side and a scattered series on the outer; in the mass of parenchyma within this ring of sieve-tubes a sclerotic group, surrounded by an endodermis, appears; the arched xylem of the leaf-trace separates from the xylem of the stele at one end first, and, through the gap formed, the external phloem becomes continuous with the internal in the manner seen in *G. dicarpa* (Fig. 21, to be described later); then a stage, resembling that shown for *G. dicarpa* in Fig. 22, is reached, though in *G. flabellata* the bending in of the external endodermis round the sclerotic group takes place when the xylem and phloem of the other end of the petiolar bundle have become separated from the corresponding tissues of the stele. This point is connected with the bundle not swinging round so far as it does in *G. dicarpa* before becoming free. The type of structure now attained, viz. an arched bundle with an intrusion of endodermis and cortical sclerenchyma in its concavity, is continued in the petiole.

For *Eugleichenia* Poirault gives diagrams (Figs. 13 and 14) of the nodes of *G. Boryi* and *G. dicarpa* (under syn. *G. hecistophylla*). In *G. Boryi* one may summarize his description

thus:—A nodal island consisting of parenchyma and a ring of sieve-tubes appears in the xylem, but contains no sclerotic tissue or endodermis; the arch of xylem outside this with three protoxylems separates for the leaf-trace, while the phloem of the nodal island becomes continuous with the outer phloem of the stele, part of it then apparently passing out as the internal phloem of the leaf-trace, and part remaining to repair the gap in the phloem of the stele. Apart from the absence of sclerotic tissue and endodermis here, the structure is not very different from what was found in one of the nodes of *G. dichotoma* (the fourth node described).

As a node of *G. dicarpa* showed some points of difference from Poirault's description ('93, p. 173), diagrams of the node of this species are given here. Figs. 20 and 21 show two stages, in which the xylem of the leaf-trace is preparing to separate. In Fig. 20 the phloem (*ph.*¹) has curved into the bay of the xylem. In Fig. 21 the phloem forms a wide loop in the xylem-arch, and a group of brown sclerotic tissue (*sc.*), surrounded by an endodermis, has appeared in the pericycle. In Fig. 22 the sclerotic group holds a transitory connexion with the sclerotic cortex, its endodermis becoming continuous with the outer endodermis¹. The sclerotic group then becomes shut in as it was before, and the leaf-trace becomes nipped off from the stele in the usual way, so that in Fig. 23 one sees the leaf-trace separate, and with a sclerotic group (*sc.*, which is bounded by an endodermis) in its central parenchyma (pericycle). The type of structure is identical with what is found in certain parts of the petiole of *G. dichotoma*. The group of sclerenchyma, however, is only continued for a short distance upwards in the base of the petiole, its elements becoming reduced in number till none are left, but surrounded to the last by an endodermis. Fig. 13 shows the group reduced to two fibres, which are separated by an endodermis from the surrounding, sclerotic elements of the pericycle.

¹ Neither the presence of the endodermis surrounding the sclerotic group nor the connexion of the latter with the cortex appears to have been observed by Poirault.

In the lower part of the petiole of *G. circinata* a small sclerotic group, surrounded by an endodermis¹, is found in the central pericycle, just as in the leaf-trace and petiole of *G. dicarpa* (Figs. 23 and 13). In the case examined, however, it nowhere included more than three fibres. It dies out upwards in the petiole as in the other species just quoted. A node of *G. circinata* was examined with the expectation that the sclerotic group would behave as in *G. dicarpa*, but it was found to be restricted to the base of the petiole, and to have no connexion with the cortical sclerenchyma. In the node examined, the leaf-trace separated with a closed xylem surrounding very little central parenchyma. As the leaf-trace passed through the cortex, its central parenchyma increased to a triangular sclerotic group, and within this there appeared a brown sclerenchyma-fibre, surrounded by an endodermis, just about when the petiole became free. The single fibre was soon replaced by two and then three, and these were reduced to one again, which soon disappeared. The fibres thus had no connexion with the cortical sclerenchyma, but formed a thin spindle-shaped group sheathed with endodermis and isolated in the central pericycle of the petiolar bundle. As the group is so small, and occurs within other sclerotic tissue, it is probably a reduced structure, derived from one like that found in *G. dicarpa*. Poirault gives a figure of the petiole of a variety of *G. circinata* (*G. Speluncae*), showing a central sclerotic group of thirteen elements, surrounded by an endodermis, so in this form the sclerotic tissue is better developed, and might be continued down into the node as in *G. dicarpa*, or still further into an enclosed nodal island as in Poirault's diagram of *G. hecistophylla*, and in *G. dichotoma*.

The characters of the node of *Gleichenia* may be summarized shortly: in several species a parenchymatous nodal island is formed in the xylem of the stele, and contains phloem and a group of sclerenchyma (surrounded by endodermis); the sclerenchyma is continued for a short distance

¹ This endodermis contains granular (probably siliceous) bodies exactly similar to those occurring in the external endodermis of the bundle in this species.

upwards inside the bundle of the petiole (*G. dicarpa*), or to the top of the petiole (*G. dichotoma*); it has no connexion with the cortex (*G. dichotoma*), or a transitory connexion with the cortex just before the separation of the leaf-trace (*G. dicarpa*), or becomes continuous with the cortex when the leaf-trace separates, and is not afterwards continued inside the leaf-trace (*G. flabellata*, &c.); but in this case it may perhaps be represented by the sclerotic tissue filling the concavity of the bundle, but external to the endodermis of the latter.

Poirault's comparison of the nodal structure of *Gleichenia* with that of the young seedling stem of *Trichomanes* appears to be based on a misapprehension with regard to the structure of *Trichomanes*, as pointed out under the Hymenophyllaceae (Boodle, '00, p. 493).

. GLEICHENIA, rachis and pinnules.

In the primary and secondary pinnae at their bases and for some distance upwards, and also in the primary rachis, the vascular bundle has the same type of structure as in the petiole of the same plant, though it is of smaller size.

In *G. dicarpa*, var. *longipinnata*, the separation of the bundles for the first two primary pinnae takes place in a simple manner. The C-shaped band of xylem becomes buckled in at two points so as to form three contiguous arches (standing on the same plane), which then separate and diverge each invested by the other usual constituents of the bundle, so that three separate and nearly similar bundles are produced. The two outer ones pass out to the first pair of pinnae, while the middle one is continued straight on into the main rachis. This division of the petiolar bundle into three takes place close below the point of insertion of the pinnae.

G. cryptocarpa has been mentioned before for the exceptional feature it presents in the division of the bundle into three close to the base of the petiole. Here also the mode of separation appears to be simple. The whole bundle, as well as the

xylem, is C-shaped, and divides into three separate arched bundles, probably without much previous buckling.

In *G. flabellata* there is greater complexity. The behaviour of the xylem could not well be made clear without the aid of diagrams, so the description will be restricted to the bundle, as limited by the endodermis. Near the top of the petiole the bundle becomes elongated at right angles to its median plane, so as to form a low arch with the ends turned in like a compressed C. The ends approach until they meet, when the endodermis becomes fused, so that the bundle now has a complete inner and outer ring of endodermis. The form of the bundle is now a long hollow ellipse. Two bridges of bundle-tissue are now formed across the central cavity, and the bundle becomes a chain of three loops. By splitting of the bundle through the two bridges, three closed annular bundles are produced, of which the middle one, belonging to the rachis, has an annular xylem. Before long all three bundles open and return to the typical arched form. In *G. dichotoma* the division of the bundle takes place in a somewhat similar manner.

There is the same transition to collateral structure that is found in the leaf of many other Ferns. In *G. flabellata* one of the secondary pinnae not very far from its tip had a bundle with an oval endodermis (not invaginated) and an arched xylem with three protoxylems. The form of the xylem is very similar to that found by Gwynne-Vaughan ('01, Pl. III, Fig. 8) near the tip of the rachis in *Loxosoma*. The phloem curves round the hooked ends of the xylem, so as nearly to meet; the bundle is therefore practically concentric. In the pinnules, however, the bundle of the midrib is reduced to collateral structure. The small bundle, which is circular in outline, is bounded by an endodermis, has a roughly semi-circular group of xylem, with a layer of phloem on its curved lower side, and one protoxylem-group at the middle point of its flat upper side. This again corresponds with what is found in the principal vein of the leaf-segment of *Loxosoma* (Gwynne-Vaughan, '01, Pl. III, Fig. 7, e.).

In the species examined the stomata were restricted to the lower surface of the pinnules. One interesting arrangement in the xerophytic form, *G. dicarpa*, var. *alpina*, is worth mentioning. Each pinnule was, in a sense, revolute. As seen in transverse section the right and left half of the lamina formed two semicircles springing from the midrib, and the lower surface of the midrib bore flat scales, which were spread out horizontally and reached the margins of the lamina. Consequently the lower surface of the latter, which bore the stomata, formed the lining of a chamber, to which the scales acted like a lid. The mesophyll in this variety was more lacunar than in species, which appeared less or scarcely xerophytic. The upper epidermis was also thicker-walled.

GLEICHENIA PECTINATA.

This species differs from all those described above in possessing solenostelic structure. Figs. 24-27 illustrate the internodal and nodal structure of *G. pectinata*. Only dried material was obtainable, which accounts for the collapsed condition of most of the soft tissues. Fig. 24 is a photograph of a transverse section of the stele in the internode. The position of the protoxylems, inner and outer phloem, &c., is shown in the diagram of the same section (Fig. 25). The phloem on both sides of the xylem is mostly crushed, but here and there patches of it were in fairly good condition, and when examined under the high-power showed elements, which in the characters of their refractive walls, sieve-plates, &c., were typical Fern-sieve-tubes. It was in the internal phloem of this species that callus-knobs were recognized by using the azo-blue test. The central space in Fig. 24 was due to the inner endodermis having become torn, and the central sclerenchyma having dropped out. The xylem contains very large scalariform tracheides, and these are separated from one another by chains and groups of parenchymatous cells. The xylem is mesarch with, at this point, 8-9 groups of spiral protoxylem-elements. The structure is essentially the same as in other species of *Gleichenia* possessing a lobed xylem,

but with the remarkable difference, that here the more central part of the xylem is replaced by a mass of sclerenchyma surrounded by a ring of endodermis (Fig. 25, *i.e.*), pericycle, phloem (*i.ph.*) and conjunctive parenchyma. The upper part of the xylem has a rounded surface; in the lower part the lobes correspond with the protoxylem-groups. One of the groups, however, appeared to have split into two, probably as a stage preliminary to the production of a root. A point to which we shall return is the occurrence of a few fibres in connexion with one or more of the protoxylems. The stele of *G. pectinata* is larger than that of any other species examined, the diameter of its xylem being $1\frac{2}{3}$ mm., while that of *G. pubescens* is $1\frac{1}{3}$ mm. The larger forms of *G. longissima* could not be examined, but they would probably have a stele at any rate as large as that of *G. pectinata*, and might possibly also show solenostelic structure.

A section through the nodal region is seen in Fig. 26. The lower part of the stele remains as in the internode; the central sclerotic tissue is *in situ*, and has a two-armed upward extension, which fills the concavity of the band of tissue preparing to separate as a leaf-trace. At this point the whole mass of sclerenchyma has a form resembling a T, or perhaps still more like a longitudinal section of a mushroom. In Fig. 27 the leaf-trace has become free, and is on its way out through the cortex. The stele is left open by a leaf-gap, so that both its central sclerenchyma and that of the leaf-trace are continuous with the cortical sclerenchyma.

The petiolar bundle resembles the leaf-trace, but has a nearly circular external outline, broken of course at the point where the T-shaped promontory of cortical sclerenchyma passes into its interior. The xylem has the usual arched form, has one protoxylem in each of the two hooks of the xylem, and about ten other groups. Fibres are found in the slight bays of the hooks, and are scattered at intervals on the inner side of the xylem-arch. The fibres represent phloem-elements in the lower part, but may perhaps be pericyclic elsewhere. The petiolar bundle is thus like that

of other *Mertensias*, e.g. the form of *G. longissima* described above, though smaller than in the latter.

To return to Fig. 26 for some details connected with the separation of the leaf-trace, there is a protoxylem-group in the xylem just below the tip of each arm of the sclerenchyma. Phloem is of course found surrounding the whole of the xylem, and, on the inner side of the latter, it is found as a continuous layer, interrupted only below the middle region of the arch of xylem destined for the leaf-trace. Of the phloem-elements present within the lower parts of the xylem-arch a few are transformed into fibres, and the latter are also found in the phloem between the tip of each arm of the sclerenchyma and the adjacent protoxylem-group, already mentioned. This protoxylem forks into two, of which one group goes to supply the future hook of the leaf-trace, while the other one remains in the stele, still with the phloem containing fibres between it and the sclerenchyma. Then a bar of xylem is formed cutting across the arm of sclerenchyma near its tip; the latter having become previously elongated as on the right-hand side in Fig. 26. In this way a piece of brown sclerotic tissue (apparently surrounded by an endodermis) comes to be enclosed in an island in the xylem, in which also phloem is found, though chiefly represented by pale-walled fibrous elements, and this island is adjacent to a protoxylem-group. Next the bar of xylem splits, so that the xylem of the leaf-trace is separate from that of the stele, then phloem, endodermis, &c., appear in the gap so that the end of the leaf-trace becomes free from the end of the solenostele. The above stages do not take place simultaneously at the two ends of the leaf-trace, as it swings round slightly so as to pass off obliquely. In Fig. 27 two dark patches are seen immersed in the xylem of the open solenostele near its two ends. These are the two islands, whose formation has just been explained. They contain brown sclerenchyma, parenchyma, and phloem-fibres. The brown fibres in the two islands are not continued above the node for any great distance after the separation of the

leaf-trace, but the phloem-fibres are found after the solenostele has become closed. In this node moreover a few phloem-fibres occurred in connexion with the two protoxylem-groups next below those concerned with the leaf-trace, but the protoxylems in the lowest pair of xylem-lobes (in Figs. 26 and 27) were not accompanied by any such fibres. From this it appears that the phloem-fibres may be continued (as in this specimen) from one node to the next, and that the two lowest lobes of the xylem serve for the attachment of roots only, while all the other protoxylems are connected with leaf-traces, which are therefore probably tristichously arranged, the node described being one that bears a median leaf. Fig. 28 represents a scalariform tracheide of this species, and shows a peculiarity in the arrangement of the pits, which is rather common here.

GLEICHENIA, root.

The roots of *Gleichenia* are commonly tetrarch, and rootlets of *G. circinata*, var. *longipinnata*, were found to be diarch and triarch. Russow ('72, p. 97) describes the roots of *G. polypodioides* and *G. glauca* (= *G. polypodioides*, Sm., or perhaps *G. longissima*, Bl.) as being triarch and pentarch respectively. The stele of the root of *G. circinata*, var. *microphylla*, is represented in Fig. 29. The four protoxylem-groups are seen in contact with the pericycle, which varies from one to two cells in thickness. Only one of the four phloem-groups is shown in the drawing; its protophloem is at *pph*. Two of the large scalariform tracheides are incompletely differentiated (*y.t.*). A root of *G. dicarpa* had a structure, which at first sight appeared to be normally triarch, but, although there were only three groups of phloem, one of the protoxylems was split into two groups of small peripheral tracheides, which were separated by two parenchyma-cells. This structure probably signified that the root was tetrarch in one region and triarch in another.

At any rate in certain cultivated specimens, arrested roots

occur rather frequently. A root-stele passes through the cortex of the stem, and enters a short spine-like projection, which is the free part of the root. As the external tissues at its tip become sclerotic, it appears to be incapable of further growth. The stele in this spine and the root-trace in the cortex of the rhizome were found to be tetrarch in *G. dichotoma* and *G. dicarpa*, but differed from the normal roots of these species in having a tendency to form a pith. Thus in an arrested root of *G. dicarpa* the four xylem-groups did not meet at the centre but abutted on a small pith. A section through the trace of this root in the cortex of the rhizome showed a similar structure, except that one of the pith-cells near the centre was replaced by a tracheide, while two or three others appeared to have begun to thicken their walls and then to have undergone partial mucilaginous degeneration. Probably, when the arresting cause affected the organ, no further tracheides were differentiated, and consequently in the free part of the root the elements in the central part of the stele, which normally would have developed as tracheides, remained parenchymatous to form a pith. In an arrested root of *G. dichotoma* most of the central tracheides had been formed, both in the root-trace and in the spine.

These points are mentioned partly as an analogy illustrating what may perhaps have taken place in the stem of certain Ferns, and led to the production of a pith. In this connexion it is as well to refer to the occasional occurrence of half-thickened and partly collapsed or even scarcely thickened elements, evidently representing tracheides. They were found sometimes as a group of two or three in the xylem of otherwise typical roots, and also near the centre of the xylem in the rhizome in specimens of three or four species. They sometimes also occur in petiolar bundles, and are apparently quite unconnected with any external mechanical injury. They may perhaps be due to the plant having grown, whether in the open or in cultivation, under conditions differing from those of the normal habitat of the species.

In species with a lobed xylem-mass in the rhizome, the

roots are inserted on the lower and lateral lobes. If one follows one of these lobes in the acropetal direction towards the insertion of a root, one finds that it becomes more and more prominent (Fig. 14, *r.*), and finally the outer part of the lobe separates as the root-stele. The latter passes out slowly, making a very acute angle with the stele until it has passed through the pericycle, but as it enters the cortex, it usually curves more sharply and soon turns outwards horizontally, i. e. radially in a transverse plane.

In *G. dichotoma* an attempt was made to determine the origin of the protoxylems of the root. Though the point was not established with certainty, it appeared that two protoxylems of the root-trace were supplied from the protoxylem of the xylem-lobe, and that the remaining two originated independently, during the passage of the root-trace through the cortex.

GLEICHENIA, seedling.

A series of microtome-sections was made of a seedling of *G. circinata*, var. *microphylla*. Although the soft tissues became rather shrunk in the process of embedding, the structure could be fairly well determined.

The primary root has diarch structure. In the region of the foot, the root-stele curves towards the prothallus, becoming horizontal, and then vertical again. It then still has diarch structure, but in passing upwards the protoxylems become less evident, and the xylem-plate gradually becomes broader. The first leaf-trace, containing only a few tracheides, is given off from one of the ends of the xylem, corresponding in position to one of the root-protoxylems. The xylem by this time has a narrow elliptical outline, but rapidly approaches oval form, while one or two parenchymatous cells appear amongst the tracheides, soon after the first leaf-trace has become free. The first root-trace is attached somewhat laterally to the xylem, and shortly afterwards the second root-trace is inserted about opposite the first one. The parenchyma-cells in the xylem have increased in number,

and some have arranged themselves in chains, the external cells being in contact with the conjunctive parenchyma bounding the xylem. They become more scattered again, the second leaf-trace is given off about opposite the first one, and a little higher up the third root is attached. The xylem of the stem is now roughly circular in outline, and consists of tracheides of fairly uniform size (probably all scalariform) and a few xylem-parenchyma-cells amongst them. Thus, except for the absence of protoxylem-groups, the type of structure of the mature stem is already attained. Where the third leaf-trace was given off, the stele was incompletely differentiated, and, at a short distance above, became procambial. It is a pity that no older seedling was obtainable, but as a structure practically resembling that of the mature stem of *Gleichenia* was arrived at without any more complicated stage being passed through, it seems probable that such complication (e. g. solenostely) would not be found higher up. Thus, as far as the data go, the seedling-structure gives no evidence of the protostely of *Gleichenia* being due to reduction. The petiolar bundle of the third leaf has a small band of about seven tracheides, and apparently has phloem on the outer side only, i. e. it is collateral.

PLATYZOMA, habit.

The stem of *Platyzoma microphyllum*, which is the only species of its genus, is a creeping rhizome, with roots on its lower side. It differs from the rhizome of *Gleichenia* in bearing densely crowded leaves, which are not restricted to the upper side of the stem, but have polystichous arrangement. The leaves are 12 in. or less in length, and are simply pinnate. The pinnae are very small (about 2 mm. in length), ovate-orbicular in outline, and have their margins revolute as in *Erica hiemalis*, so that the stomata, which are restricted to the lower side, are placed in a nearly closed cavity. The plant is evidently strongly xerophytic, and the leaf has no doubt become simply pinnate by reduction. As mentioned

by Bower ('99, p. 32), the leaf is occasionally forked, which points to its having been reduced from a dichotomous type, such as is found in *Gleichenia*.

PLATYZOMA, structure.

In *Platyzoma microphyllum*, as the leaves are crowded, numerous leaf-traces are seen in the cortex of the rhizome in any transverse section. The leaf-traces show spiral seriation, as is seen in Fig. 30. This was drawn from a section, which included rather less than half the cortex—the part with bundles on the right; the dotted outline of the remainder of the cortex is added to complete the diagram. Fig. 31 is a diagram of the stele, giving the position of the outer endodermis, phloem, xylem and inner endodermis. Fig. 32 is a photograph of about half the stele. On the outside of the figure are the pericycle (3–4 cells thick) and the phloem (*ph.*) (seen in places as a dark line), each forming a continuous zone. Within these is the xylem, which resembles that of *Gleichenia* in being composed of scalariform tracheides together with chains and groups of parenchymatous cells, but differs from the xylem of all the species of *Gleichenia* except *G. moniliformis* in having no definite protoxylem-groups. The smallest tracheides occur in the outer region of the xylem, as seen in Fig. 32, so one may assume that the protoxylem-elements are distributed at or near the periphery. They are scalariform, and both in this respect and in being scattered at the periphery they resemble the corresponding elements in *Lygodium*. On the other hand the structure of the stem resembles what is found in *G. pectinata*, in that the xylem forms a ring, and that within it there is an inner endodermis enclosing a central mass of sclerenchyma. Of course it differs from that species in the absence of internal phloem, and of definite groups of spiral protoxylem-elements. The inner endodermis has its radial walls ruptured at most points in the photograph (Fig. 32, *i.e.*), and the central sclerotic tissue comes out as a dark mass. Its walls were not

very strongly thickened in this specimen, but were brown, and its elements contained a mucilaginous substance, which was stained deeply with haematoxylene. The phloem contains typical Fern-sieve-tubes.

Figs. 33 and 34 illustrate the mode of separation of the leaf-trace. A small peripheral tangential band of tracheides becomes marked out by the appearance of a group of parenchyma in the xylem below it (Fig. 33). In this section the group of parenchyma is unusually large, and happens to fit on to a chain of xylem-parenchyma (*p.r.*), which is radially placed. The band of xylem mentioned above becomes free as the xylem of the leaf-trace. It may pass off straight, or may swing round slightly before separating. In Fig. 34 the same leaf-trace (*l. t.*) has reached the outer limit of the pericycle. It consists of the band of xylem and an arc of phloem, detached from that of the stele, which has already repaired the gap. After the leaf-trace passes the endodermis of the stele, it is invested with a complete endodermis. As it passes through the cortex of the stem, it remains collateral, its xylem becomes crescent-shaped, and acquires two protoxylem groups on its inner side, not far from its ends. No median protoxylem-group could be recognized. No leaf-gap affecting the endodermis is present, and frequently the exit of the leaf-trace scarcely affects the xylem of the stele, beyond leaving a small external pit like that seen in Fig. 32. Figs. 33 and 34 were drawn to show the nearest approach to a true leaf-gap met with. Longitudinal sections through the insertion of a leaf-trace did not show any passage of leaf-trace-parenchyma towards the centre of the stele.

A diagram of the petiolar bundle is given in Fig. 35. It has an arched xylem with protoxylems on the inner face at the two sharp bends. The tissue within the arch appears to consist chiefly of phloem.

In the rachis the xylem-arch is converted into a nearly straight band with a protoxylem immersed in it near each end. A little phloem is still found on the upper side, besides the arc of it on the lower. There is still no median protoxylem.

In the pinnae, which probably represent the pinnules of species of *Gleichenia*, the upper epidermis is thick-walled, and the mesophyll lacunar. The form &c. of the pinnae has been described in the section on the habit.

The roots are diarch.

CONCLUSIONS.

Owing to lack of space, general considerations on the structure of the *Gleicheniaceae* must be treated very shortly in the present paper.

Two possibilities must be kept in view: (1) That the protostelic condition of most *Gleichenias* is primitive and that the solenostelic type of *G. pectinata* has been derived from it; or, (2) that the protostelic *Gleichenias* owe their structure to reduction from solenostely. Taking the second assumption, the nodal islands of the protostelic *Gleichenias* might be regarded as ancestral remnants of a previous solenostelic structure. On the first supposition, on the other hand, the nodal islands might be looked upon as local complications of the stele, directly connected with the insertion of the leaf-trace. The further theory may be added, that the solenostelic structure of *G. pectinata* may have originated through the formation of a more bulky nodal island (containing phloem, sclerenchyma, &c.) and the continuation of the structure found in it through the internode. This is practically similar to the explanation given by Poirault ('93, p. 182) of the origin of the structure in *Platyzoma*. The node is often regarded as being prone to show ancestral characters. This may be true in certain cases, and the reverse in others. The importance of the effect of the leaf in moulding the structure of the stem has been pointed out by Jeffrey ('00) and by Gwynne-Vaughan ('01, p. 87). The requirements to be fulfilled in conduction and mechanical support would be somewhat more complicated at the node than in the internode, and might well lead to greater complexity in structure at the node, so that new types of structure would be liable to appear there. If the

new structure were suitable for the internode, it might spread through that region, by what might be called transformation of the tissues there. On the other hand, if reduction should take place in the structure of the internode owing to change in habit or other causes, a vestige of the previous structure might be retained at the node, as being more suitable to the slightly different requirements of the latter. The nodal island in *Gleichenia* may therefore be looked upon as either a remnant of previous solenostelic structure, or as a local modification in the stele, which finally led to solenostely in *G. pectinata*. The latter view on the whole appears the more probable. It is favoured by the two facts that the seedling-stem up to the third leaf shows protostelic structure, and that the leaf of *G. pectinata* does not carry dichotomy so far in the ramification of its frond as the other species, dichotomy in the leaf being taken as a primitive character. Much weight must not be laid on either of these features, but they are of some value, as nothing important has been noticed pointing in the other direction. In this connexion it is very interesting to note that the sorus of *G. pectinata* has unusual characters, which it shares with *G. dichotoma*, and in which these two species differ from the others of the genus, as described by Bower ('99, p. 33), namely, that it consists of more numerous sporangia. The latter are multiseriate in the sorus, and in this respect approach other Leptosporangiate Ferns (Bower, '99, p. 34). Though, considering the direction of specialization in the Leptosporangiate Ferns, the change to multiseriate sori may be taken as an advance, the uniseriate sorus might in certain cases be due to reduction, so the evidence of the sorus does not materially help in solving the present question.

It will be seen from what has been said above that the following suggestions must be put forward in a quite tentative manner. The protostelic structure of *Gleichenia* may be regarded as primitive, and the solenostelic type of *G. pectinata* as derived from it, the nodal islands of the protostelic forms representing an intermediate step. *Platyzoma* appears to be a xerophytically reduced form, in which the leaf-traces have

become small and crowded; it is perhaps probable that it may have been derived from a solenostelic form, by obliteration of the leaf-gaps, and disappearance of the internal phloem of the stele. This view is, I believe, held by Dr. E. C. Jeffrey. It is possible on the other hand that *Platyzoma* may have been derived from a protostelic *Gleichenia*, and its structure might then be due to the formation of a pith and internal endodermis. Although the nodal island in *Gleichenia* is here held not to be a remnant of solenostelic structure, some forms, e. g. *G. circinata*, seem to show reduction of the nodal island.

The two small nodal islands of *G. pectinata* further complicate the question. Assuming that they do not represent the nodal island of other *Gleichenias* split into two, but that the solenostelic structure is due to extension of the original nodal island, a similar extension (accompanied by fusion) of the small nodal islands of *G. pectinata*, so as to assume tubular form, would produce a double vascular ring like that described by Seward ('99) in his interesting paper on *Matonia pectinata*. This plant is perhaps not so very far removed from the Gleicheniaceae in its affinities.

Shortening of internodes and decrease of rate of growth of the stem are probably two factors, which both tend to abolition of spiral protoxylem elements. This is illustrated by *Platyzoma* and *G. moniliformis*, where spiral elements are absent, and both factors are likely to have been brought into play by the strongly xerophytic conditions, of which the habit and habitat of the two plants give evidence. The shortening of the internodes is not so manifest in *G. moniliformis*, but the change to its upright stem from the elongated rhizome of other species would probably greatly favour it.

One or two structural characters in the Order have a bearing on stelar theories. The sclerotic tissue with its endodermis in the nodal island and in the leaf-bundle is no doubt a homologous structure in the different species. Yet it may be connected with the cortex in some species, and not in

others, so that continuity of tissue is of no value in this case for determining its morphological nature. Again, it seems probable that the phloem-fibres which accompany certain protoxylem-groups through the internode in *G. pectinata* have been formed by transformation of some of the parenchyma usually found in that position, and the same might apply to the island of sclerenchyma, which is, however, only continued for a short distance above the node.

The morphology of stelar structures must remain at present doubtful, but the Gleicheniaceae have afforded no evidence disproving the general position taken in the section on the stele in the paper on Schizaeaceae (Boodle, '01, p. 403). In that paper the author is sorry to find that he misquoted Mr. Gwynne-Vaughan, by stating ('01, p. 405) that he had applied the term phloeoterma to the endodermis, and takes this opportunity of offering him an apology.

While not laying too much weight on the importance of structural differences, it may be said that the anatomy supports several points in Hooker and Baker's classification of the Gleicheniaceae, e.g. the inclusion of *G. moniliformis* in *Gleichenia*, perhaps the separation of *Platyzoma microphyllum*, and the raising of *G. pectinata* to a distinct section of the genus.

A discussion of the relation of the Gleicheniaceae to the Hymenophyllaceae and Schizaeaceae must be deferred, but it may be said here that anatomical characters rather point to *Lygodium* as being the most primitive type found within these three Orders, to the Gleicheniaceae standing higher and having been derived from some form resembling *Lygodium*, while the Hymenophyllaceae may well have been derived by specialization from some stock not far removed anatomically from the Gleicheniaceae. This agrees well with the positions assigned to these Orders on other grounds by Bower ('99, p. 129).

SUMMARY.

1. Two chief types of stem-structure are found in the genus *Gleichenia*: (a) protostelic, (b) solenostelic. The latter has only been met with in one species, *G. pectinata*. The genus *Platyzoma* exhibits a third type: (c) medullate stele, with annular xylem and internal endodermis.

2. The xylem is mesarch with distinct groups of spiral protoxylem except in *G. moniliformis* and in *Platyzoma*. No spiral protoxylem was recognized in either of these, but in *Platyzoma* some of the smaller, sub-peripheral, or peripheral scalariform tracheides quite possibly represent scattered protoxylem.

3. As pointed out by Poirault, the bundle of the petiole is rounded in *Eugleichenia*, and nearly always arched in *Mertensia*, but *G. dichotoma* forms an exception. General sclerosis of the pericycle in the petiole may occur in both subgenera.

4. In all cases a single leaf-trace enters the petiole, and usually divides into three, close below the first pinnae; in one species this division takes place near the base of the petiole.

5. The xylem in the petiole has usually the form of an arch with incurved ends. Where the bundle is small, there are one median and two lateral protoxylems on the upper side of the xylem. In larger bundles the protoxylems are more numerous.

6. In several species, at any rate, a nodal island is found in the xylem of the stele, as described by Poirault. It contains phloem and sclerenchyma; the latter being surrounded by an endodermis. When the leaf-trace becomes free, the sclerotic tissue in the nodal island may fuse permanently with the cortex, or it may have a transitory connexion with it, and then be continued for some distance upwards (surrounded by its endodermis) inside the petiolar bundle; or it may have no connexion with the cortex, although continued to the top of the petiole inside the bundle (*G. dichotoma*). The phloem in the nodal island is connected

with the phloem on the inner or upper side of the leaf-trace, but may also be continued upwards in the xylem of the stem for a short distance after the separation of the leaf-trace. In *G. pectinata* two nodal islands are found, but some of their constituent elements may be continued as far as the next node.

7. *Platyzoma* has a collateral leaf-trace but apparently a concentric petiolar bundle. The median protoxylem is absent. There is no true leaf-gap. The group of parenchyma below the separating leaf-trace contains no phloem or sclerenchyma.

8. The bundle in the midrib of the pinnules shows collateral structure.

9. The roots are mostly tetrarch. Rootlets may be diarch or triarch. The roots of *Platyzoma* are diarch.

10. The seedling stem of *Gleichenia*, except for the absence of definite protoxylem-groups, attains the typical stem-structure of the genus below the insertion of the third leaf, and by that time has given no evidence of reduction from a more complicated type.

The most important structural point met with in examining the anatomy of this Order, not previously described, is the solenostelic structure of one of the species of *Gleichenia*. Our knowledge of this type may throw considerable light on the comparative anatomy of the group.

I wish once again to express my indebtedness and thanks to Dr. D. H. Scott, F.R.S., for many valuable suggestions. I have also to thank Mr. J. G. Baker, F.R.S., Mr. C. B. Clarke, F.R.S., and Mr. C. H. Wright, A.L.S., for giving me their kind assistance in one or two points. The material used for this investigation was from plants in cultivation in the Royal Botanic Gardens, Kew, in the case of several species of *Gleichenia*; in other species and in *Platyzoma* only dried material was available. Seedling plants of *Gleichenia* appear to be difficult to raise. The one examined was grown at Kew.

REFERENCE TO LITERATURE.

Among the earlier references to *Gleicheniaceae* is one by Von Mohl ('45, p. 115), who mentions that in many thin elongated stems, e.g. *Gleichenia*, the vessels are all united into a central bundle. He also points to *Platyzoma*, previously described structurally by Robert Brown ('38-'52, p. 2), as a connecting link between the above type and that of the hollow reticulate cylinder of other Ferns. Presl ('47, p. 2) refers to the presence of only a single circular or semicircular vascular bundle in the petiole of the *Gleicheniaceae*, and gives diagrams (Pl. VI) of the petioles of *Platyzoma* and of seven species of *Gleichenia*, but in each case the bundle is simply represented by a circular outline.

Russow ('72, p. 96) states that the rhizome of *G. polypodioides* agrees in structure with that of *Trichomanes radicans*. He also describes the petiolar bundle in three species of *Gleichenia*—*G. polypodioides*, *G. dichotoma*, and *G. dicarpa*, var. *alpina* (under syn. *G. vulcanica*), and gives a fairly accurate diagram (Taf. X, Fig. 10) of the last-named variety. The only point of difference from his figure, shown by a petiole of this plant examined, was the restriction of the phloem inside the xylem-arch to the region of the hooks. The sclerotic pericycle is correctly described, and also the fibres adjoining the hooks of the xylem. Phloem-fibres are also described as being found on the outer side of the xylem at the top of the arch in this species and in *G. dichotoma*. They certainly sometimes occur in this position in both these species, but are late in differentiation and not always found. Russow makes no mention of the internal endodermis in the petiolar bundle of *G. dichotoma*.

Potonié ('83, p. 39) examined the rhizome of *G. Mendelii* (= *G. circinata*, var.), and found the typical *Gleichenia*-structure, and notes that the xylem-parenchyma (amylom of the hadrom) is a strongly developed tissue.

Thomae ('86, p. 144) remarks on the fact that in the *Gleicheniaceae* only a single vascular bundle passes into

the petiole, and mentions the occurrence of phloem-fibres and of the central sclerenchyma in the petiolar bundle of *G. dichotoma* and *G. rigida*. He gives a diagram (Pl. VII, Fig. 1 a.) of a transverse section of the petiolar bundle of *G. dichotoma*, showing the form of the xylem-arch, and three diagrams (Fig. 1, b., c., and d.) illustrating the mode of constitution of the xylem-masses of the first two pinnae and the rachis. If one bears in mind that in these diagrams it is only the xylem that is shown, and that in his description (p. 155) 'Bündel' represents 'xylem-mass,' the stages he gives may be taken as fairly accurate.

Van Tieghem ('91, p. 1376) describes the intermittent growth of the leaf in *Gleichenia*, due to the production of the dormant bud in the fork of the pinnae, and its subsequent growth as a rachis, and mentions the solid stele of the rhizome.

Poirault's work ('93) has been frequently referred to above, as he deals rather fully with the node and petiole of *Gleichenia*. In describing the rhizome of *G. Boryi* (p. 171) he speaks of the protoxylem-elements as narrow tracheides with very wide pits, so it seems that he must have missed the true protoxylem elements, which are spiral in that species, and probably in all except *G. moniliformis*. Poirault refers to the structure of *Platyzoma*, and, as mentioned above, gives an explanation of the manner in which its structure may have been derived from that of *Gleichenia*.

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EXPLANATION OF THE FIGURES IN PLATES XXXVIII AND XXXIX.

Illustrating Mr. Boodle's paper on the Anatomy of the Gleicheniaceae.

The broken lines in the diagrams represent protophloem. The following lettering is used in several of the illustrations: *e.*, endodermis; *i.e.*, inner endodermis; *x.*, xylem; *px.*, protoxylem; *ph.*, phloem; *pph.*, protophloem; *i.ph.*, inner phloem. Where *x'*, *px'*, *ph'*, *pph'* are used, they refer to the xylem, protoxylem, phloem and protophloem respectively of a leaf-trace or petiolar bundle; *sc.*, sclerotic tissue. Fig. 2 is from a photograph by Dr. E. C. Bousfield; Figs. 24, 26, 27, and 32 are also from photographs.

Fig. 1. *Gleichenia flabellata*, rhizome. Diagram of transverse section. *a.*, epidermis and outer cortex; *b.* and *c.*, middle and inner zones of the cortex respectively. $\times 9$.

Fig. 2. *Gleichenia flabellata*, rhizome. Transverse section of the stele. $\times 50$.

Fig. 3. *Gleichenia dichotoma*, rhizome. Transverse section of outer part of stele. *sc.*, innermost layer of sclerotic cortex; *s.t.*, sieve-tube; *x.p.*, xylem-parenchyma. $\times 390$.

Fig. 4. *Gleichenia circinata*, rhizome. One lobe of xylem in transverse section, showing position of protoxylem (*px.*). $\times 390$.

Fig. 5. *Gleichenia circinata* (var. *rupestris*), rhizome. Radial section of outer part of stele. *sc.*, scalariform tracheide; *x.p.*, xylem-parenchyma. $\times 390$.

Fig. 6. *Gleichenia dichotoma*, young rhizome. Transverse section of outer part of stele. *t.t.*, undifferentiated tracheides. $\times 390$.

Fig. 7. *Gleichenia dicarpa*, petiolar bundle. Transverse section. *c.p.*, cavity-parenchyma; *f.*, phloem-fibres. $\times 145$.

Fig. 8. *Gleichenia dichotoma*, petiole. Diagram of transverse section. $\times 45$.

Fig. 9. *Gleichenia dichotoma*, petiole. Diagram of transverse section of vascular bundle. $\times 45$.

Fig. 10. *Gleichenia dichotoma*, petiole. Transverse section of part of bundle, showing cavity-parenchyma (*c.p.*) and adjacent tissues. $\times 390$.

Fig. 11. *Gleichenia dichotoma*, petiole. End of central strand of sclerenchyma and adjacent tissues in transverse section. $\times 390$.

Fig. 12. *Gleichenia dichotoma*, petiole. Elements of central sclerotic strand in longitudinal section. $\times 260$.

Fig. 13. *Gleichenia dicarpa*, petiole. Two brown-walled fibres separated by an endodermis from the surrounding sclerotic pericycle. $\times 390$.

Fig. 14. *Gleichenia dichotoma*, rhizome. Diagram of transverse section of the stele in the internode. *r.*, a lobe of the xylem preparing for the separation of a root-stele. $\times 45$.

Figs. 15-19. *Gleichenia dichotoma*, rhizome. Diagrams of the stele in a series of sections through the node. *n.i.*, nodal island. The broken lines surrounding the stele show the position of the protophloem, while those in the nodal island represent all the phloem that is present there. All $\times 45$.

Figs. 20-23. *Gleichenia dicarpa*, rhizome. Diagrams of the stele in a series of sections through the node. All $\times 30$.

Fig. 24. *Gleichenia pectinata*, rhizome. Transverse section of stele. The central sclerenchyma has dropped out. \times about 18.

Fig. 25. *Gleichenia pectinata*, rhizome. Diagram of transverse section of stele. $\times 30$.

Fig. 26. *Gleichenia pectinata*, rhizome. Transverse section of stele at the node. Leaf-trace preparing to separate. $\times 15$.

Fig. 27. *Gleichenia pectinata*, rhizome. Transverse section of node. The leaf-trace has become free. \times about 6.

Fig. 28. *Gleichenia pectinata*. Part of a scalariform tracheide from the rhizome. $\times 390$.

Fig. 29. *Gleichenia circinata*, var. *microphylla*, root. Transverse section. Only one of the four phloem-groups is shown. *y.t.*, incompletely differentiated tracheides. $\times 390$.

Fig. 30. *Platyzoma microphyllum*. Diagram of transverse section of rhizome. Phloem not represented. Leaf-traces shown in the right-hand part of the cortex only. $\times 7$.

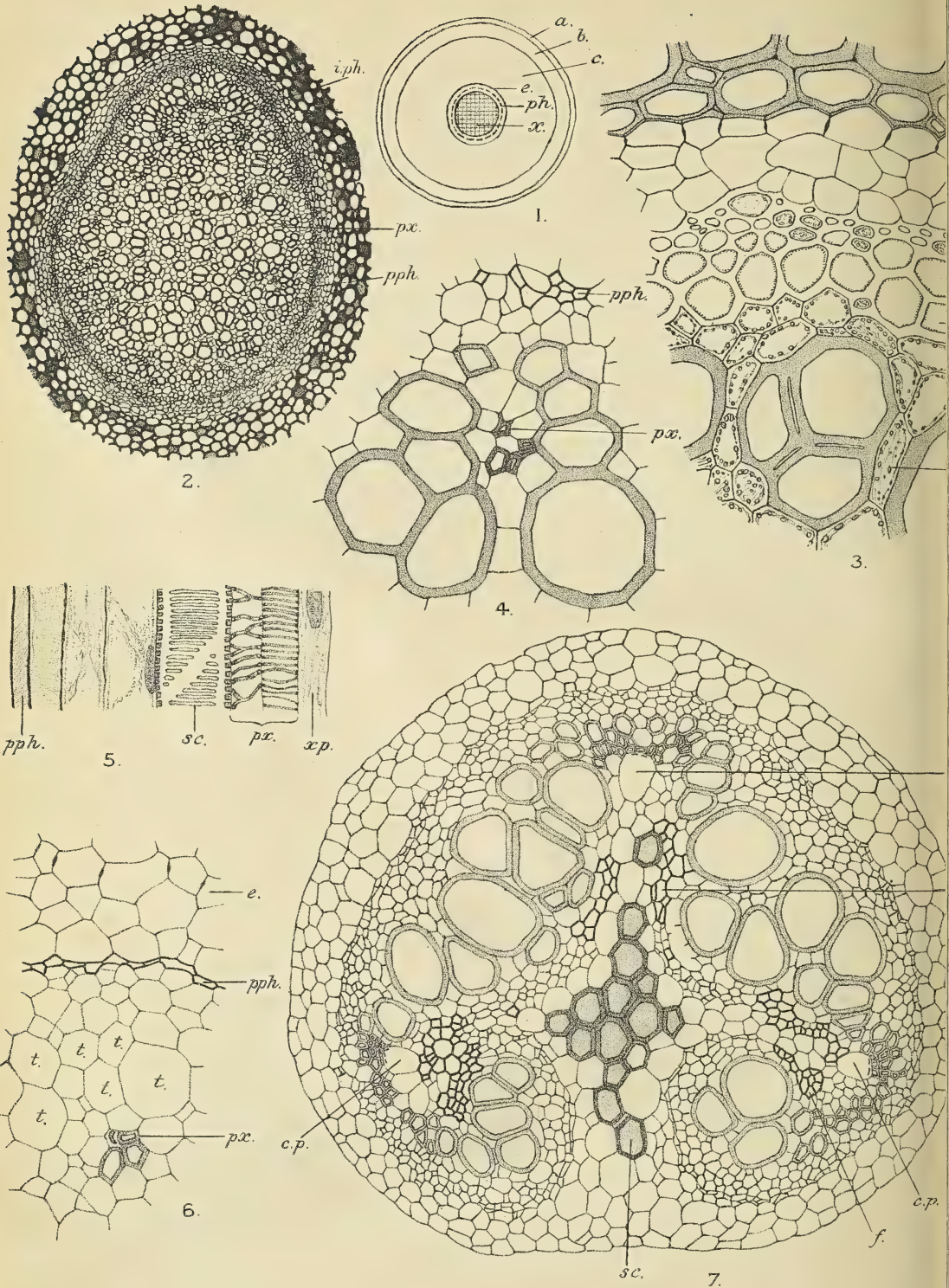
Fig. 31. *Platyzoma microphyllum*, rhizome. Diagram of transverse section of stele. $\times 30$.

Fig. 32. *Platyzoma microphyllum*, rhizome. Transverse section of about half the stele. $\times 45$.

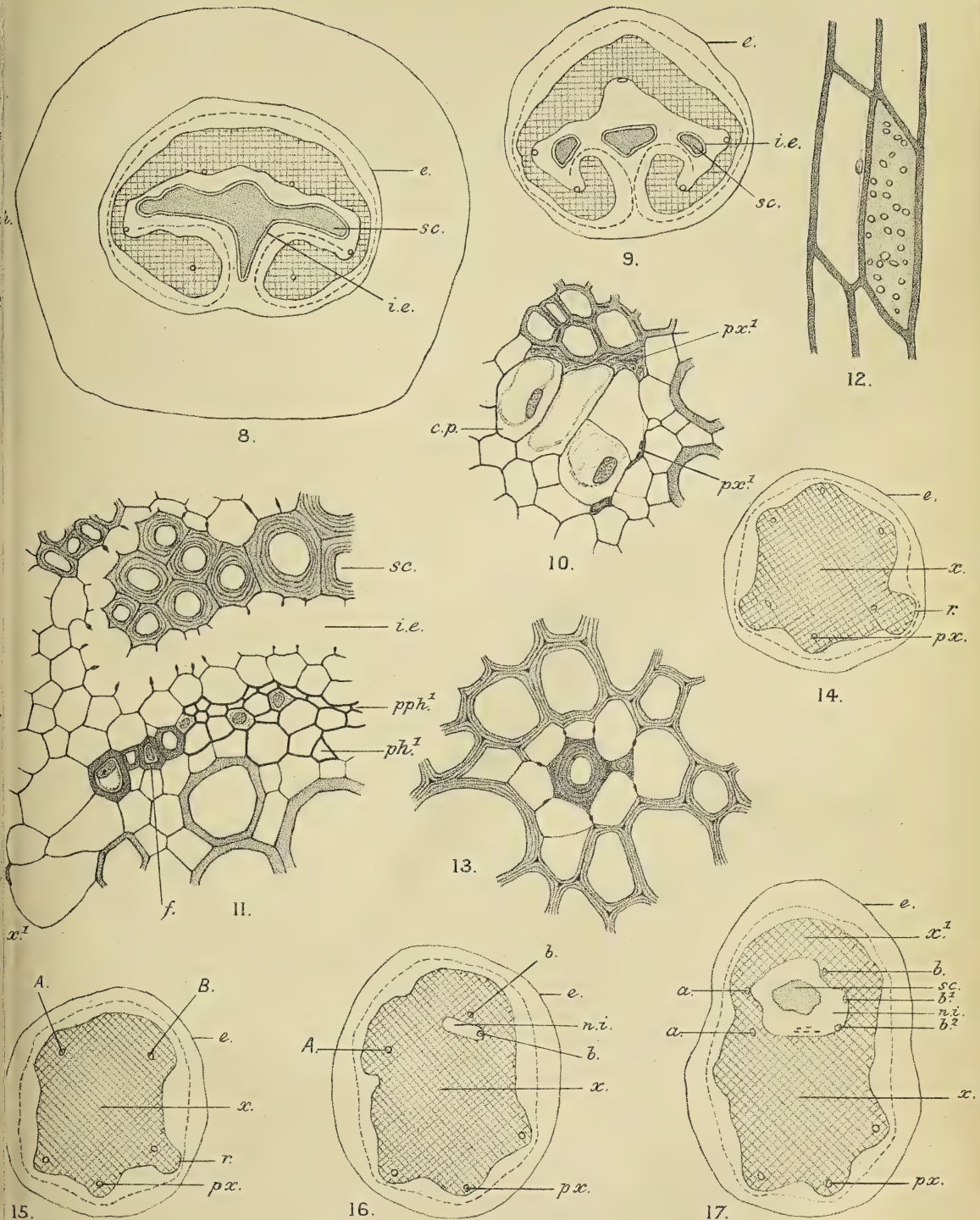
Fig. 33. *Platyzoma microphyllum*, rhizome. Transverse section, showing leaf-trace separating from stele. *p.r.*, parenchymatous ray. $\times 145$.

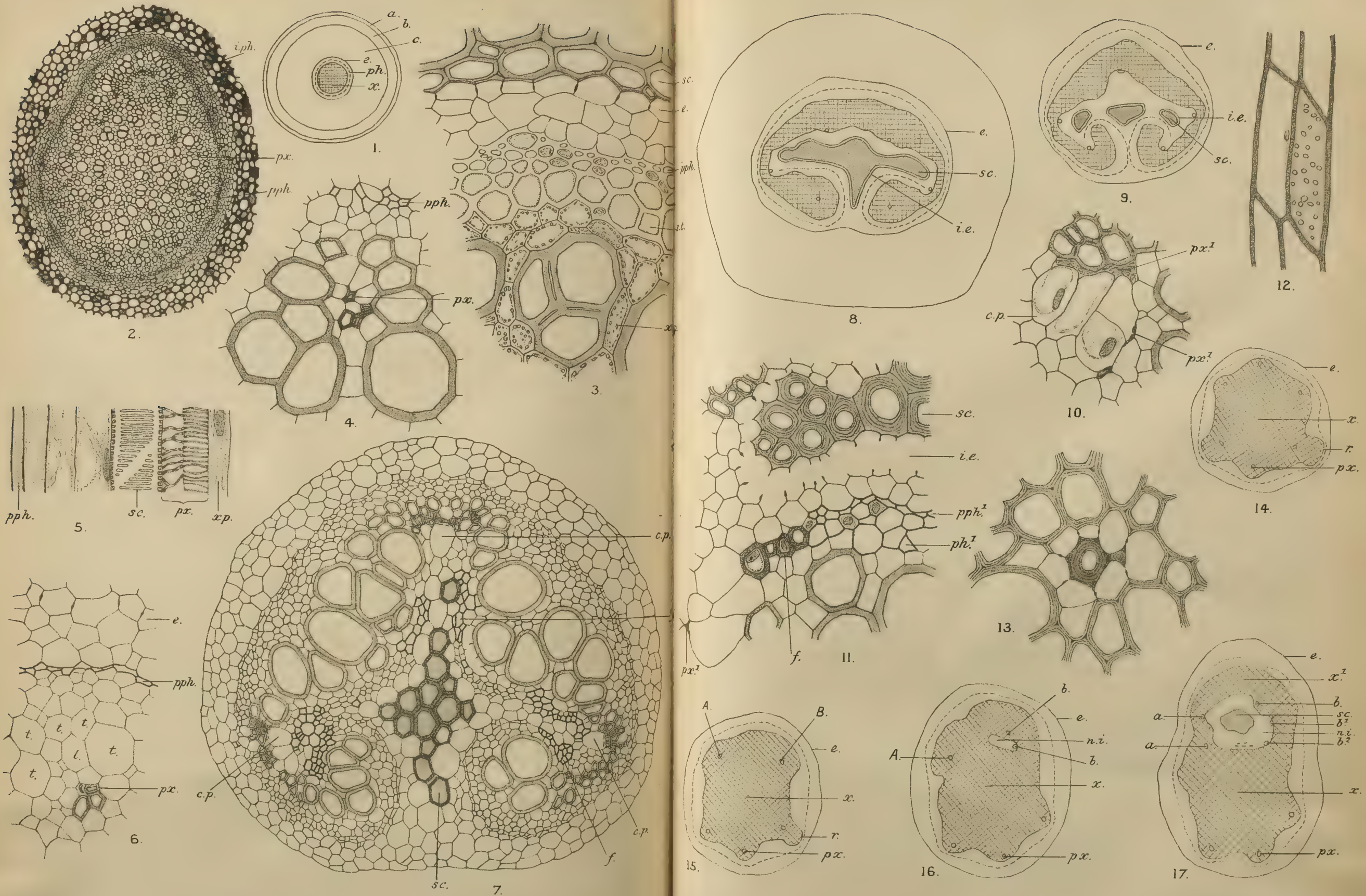
Fig. 34. *Platyzoma microphyllum*, rhizome. Transverse section, showing leaf-trace free. $\times 145$.

Fig. 35. *Platyzoma microphyllum*. Diagram of transverse section of petiolar bundle. $\times 90$.



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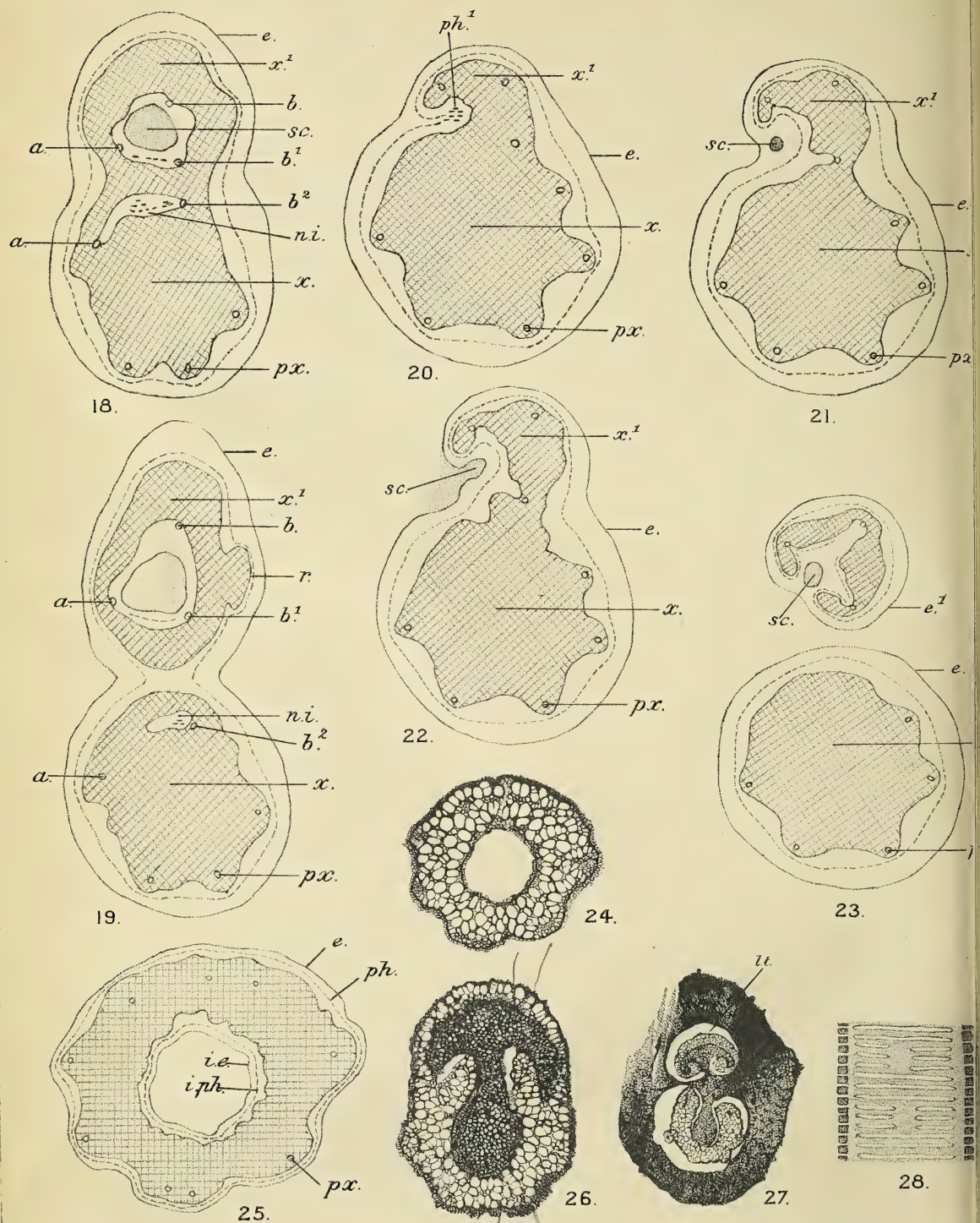




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BOODLE. — ON GLEICHENIACEAE.

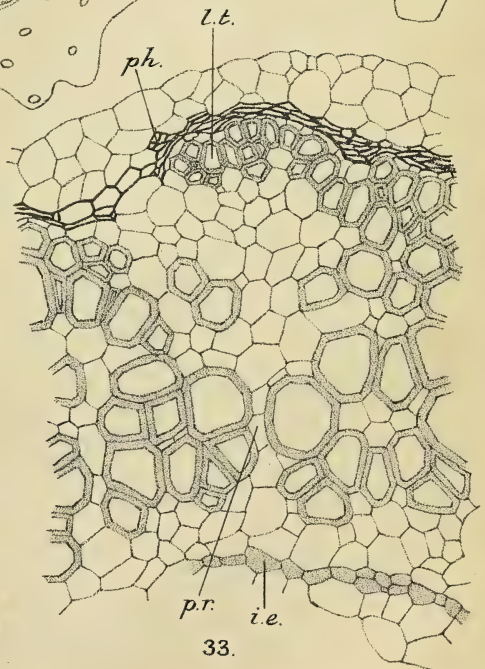
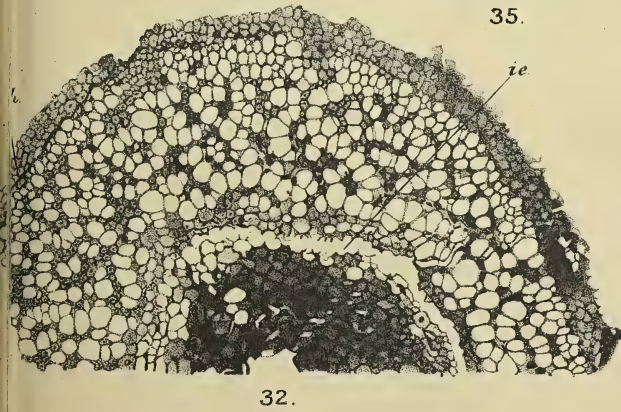
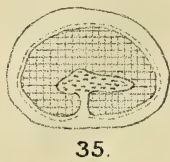
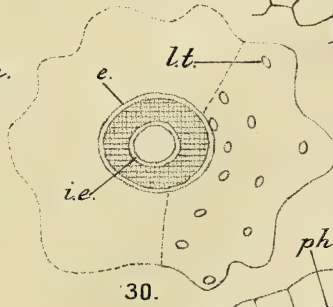
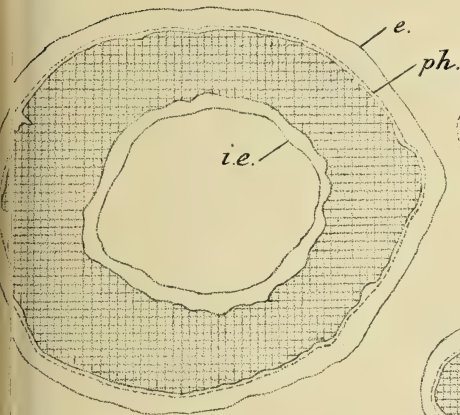
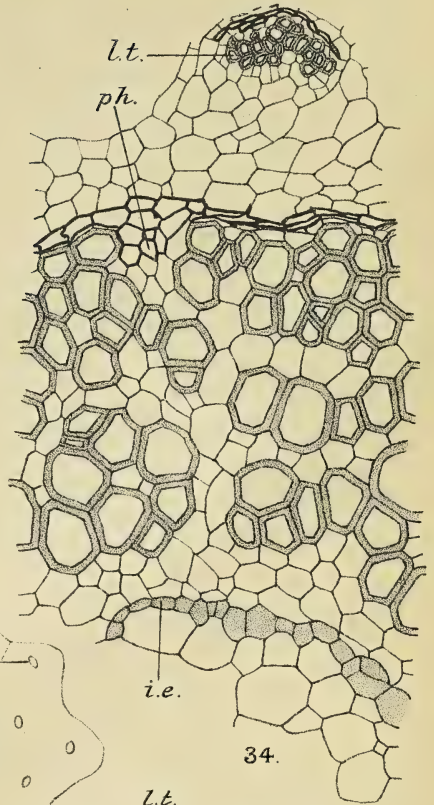
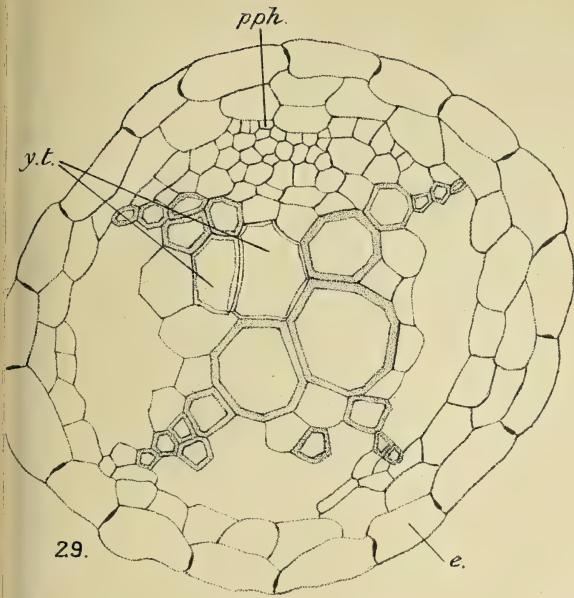
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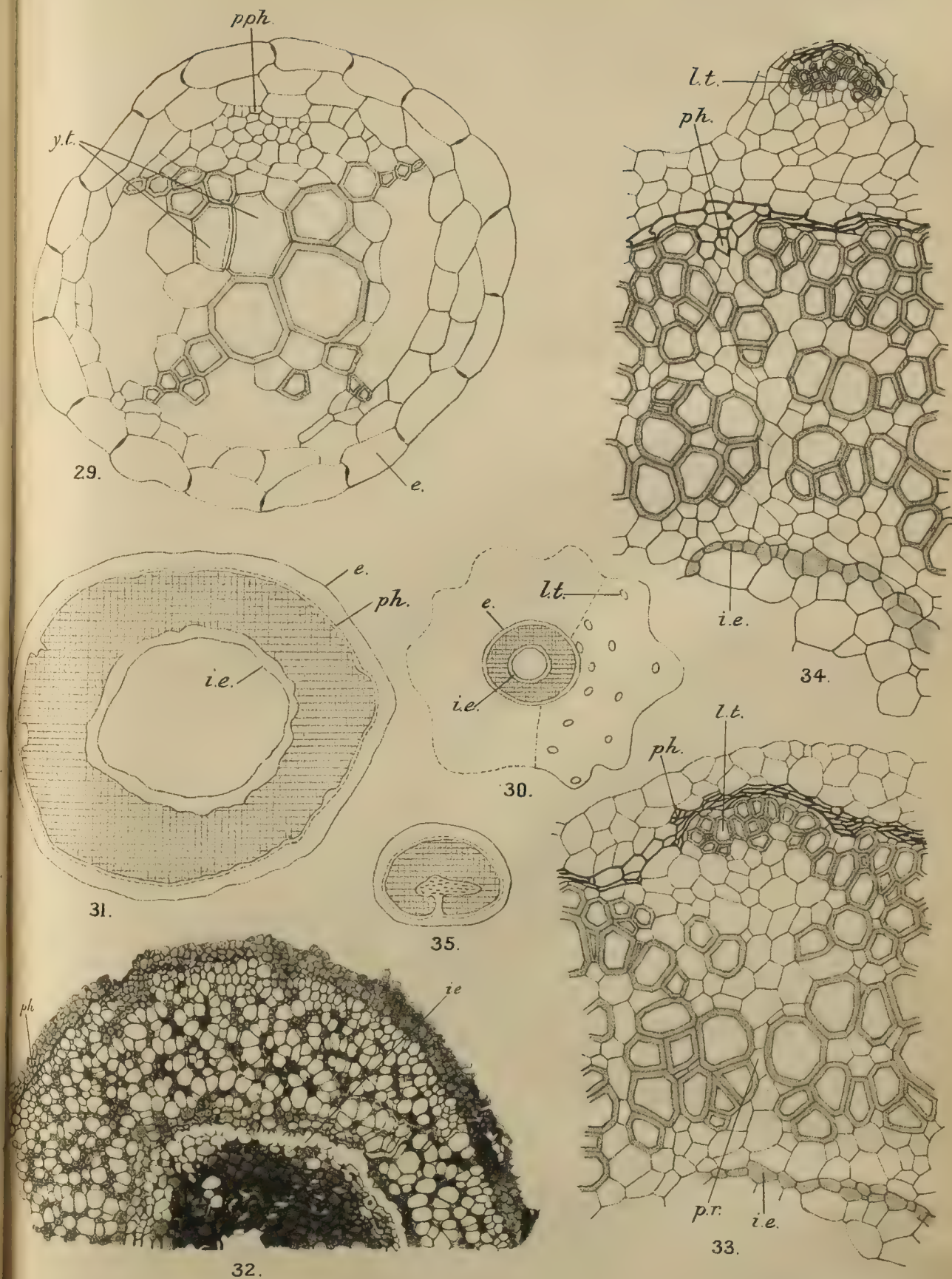
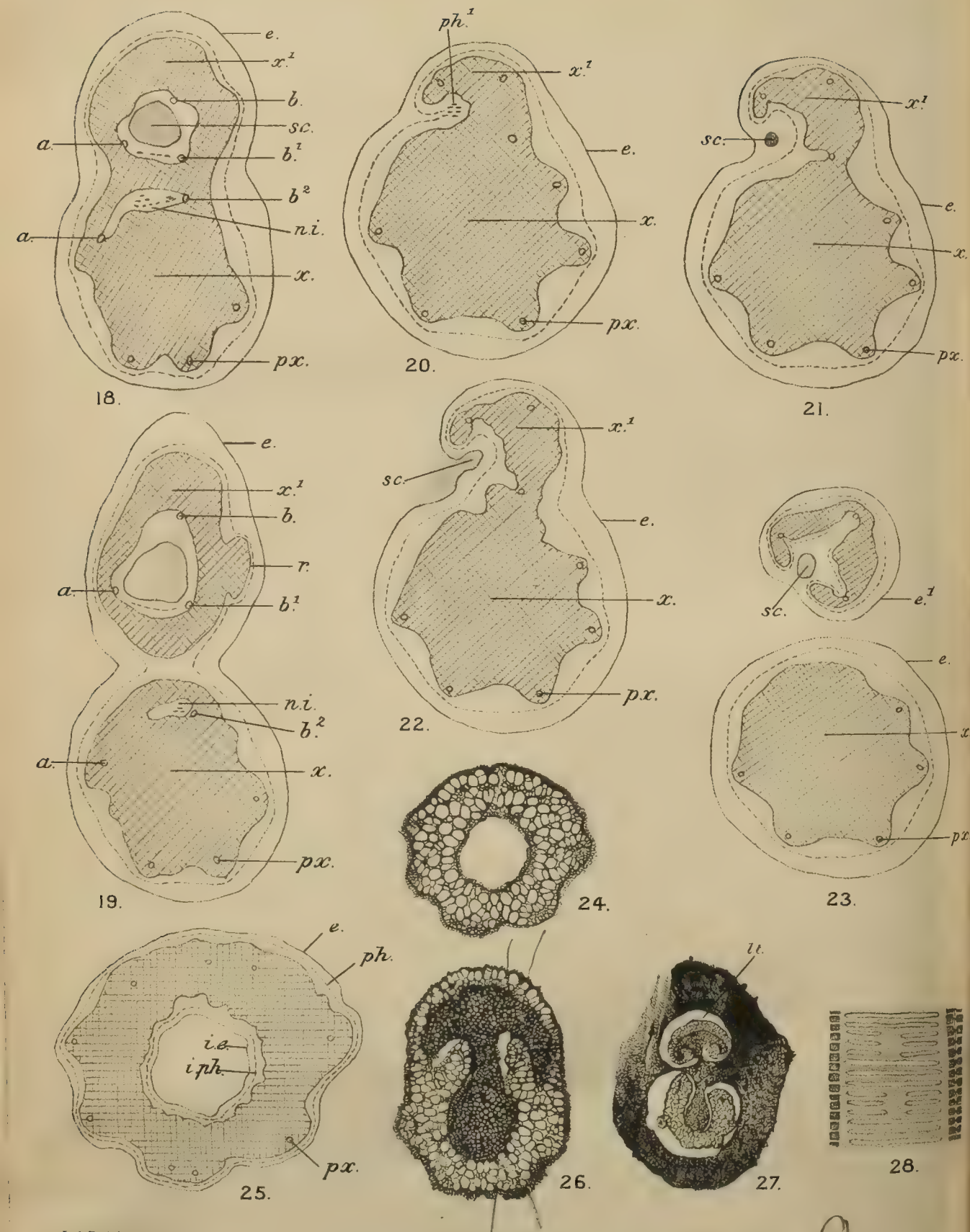


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BOODLE. — ON GLEICHENIACEAE.







Morphological Notes.

BY

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Director, Royal Botanic Gardens, Kew.

—♦—
With Plate XL.
—♦—

IV. THE HAUSTORIUM OF LORANTHUS APHYLLUS.

THE preparation described in this note has long been in my hands. There is little probability of my being able to make it the basis of a more thorough investigation. What, however, has been ascertained seems of sufficient interest to place on record.

The culture of parasitic plants, at any rate from warm countries, in Botanic Gardens is attended with obvious difficulties and has hitherto met with little success. One of the most remarkable is *Loranthus aphyllus* which grows in Chili on *Cereus Quisco*. It attracted the attention of my friend the late Professor Moseley, F.R.S., during the voyage of the 'Challenger,' as it has often done that of other travellers. On his return to England he suggested to me that it might be practicable, as cuttings of a *Cereus* can generally be rooted, to import pieces with the *Loranthus* in situ and cultivate them at Kew. Acting on this suggestion I applied to the well-known botanist, Dr. R. A. Philippi at Santiago. He took much kind trouble to comply with my request, and in 1877 sent me the desired specimen in a box.

[Annals of Botany, Vol. XV. No. LX. December, 1901.]

Unfortunately it arrived in a state of complete decay. This was not wholly a misfortune, as by placing the stem under a stream of water I was able, with a little care, to wash away the decomposed soft tissues and make a complete dissection of the fibro-vascular cylinder and of the haustorium of the *Loranthus*.

Professor Moseley refers to this in his account of the *Loranthus* in his well-known book¹:—

‘Soon after Sta. Rosa the hill-sides are seen to be covered with the tall Candelabra-like Cactus (*Cereus Quisco*). It has a most strange appearance. Other forms of Cacti, each adapted to the climate of a particular altitude, succeed one another as the slope of the Andes is climbed; those that lie highest being dwarf forms scarcely rising above the ground.

‘On the *Cereus Quisco* grows a Mistletoe (*Loranthus aphyllus*). This Mistletoe is most remarkable, because, like the plant on which it is parasitic, it is entirely devoid of leaves. It is extremely abundant, growing on nearly all the *Cereus* trees, and is very conspicuous, because its short stems are of a bright pink colour. I could not understand what it was at first, as it looked like a pink inflorescence of some kind belonging to the Cactus.

‘Mr. Thiselton-Dyer has examined the mass of parasitic tissue of this Mistletoe which draws the nourishment from the interior of the stem of the Cactus. He finds that having a soft and succulent matter in which to ramify, the basal fibres of the parasite form a large spongy mass of great size within the stem of the Cactus, which curiously simulates a mass of *mycelium*, such as produced by a parasitic fungus.’

For the comparison to a mass of mycelium Professor Moseley was responsible; in a broad sense it is not inapt. I am pretty sure that what he had in his mind was the masses of *Rhizomorpha* with which he had been much struck in the Pacific Islands.

There is a striking picture of the *Cereus* loaded with the

¹ Notes by a Naturalist on the ‘Challenger,’ pp. 544-5.

Loranthus in copious fruit in the North Gallery at Kew (No. 23). Unfortunately, as with so many other studies of vegetation by the accomplished artist, it is hung too high for proper examination.

Fig. 2 represents the appearance of the *Cereus* stem after the dissection described above. The epidermis and hypoderma have been removed, carrying with them the spiny armature and the external stems of the *Loranthus*. The cortical parenchyma and medullary tissues have been washed away, as well as any cortical fibro-vascular system which existed¹. The fibro-vascular cylinder is perforated with slits, each of which corresponds to one of the external 'spine-cushions' on the surface of the stem. In general plan the resemblance of the cylinder to that of a tree-fern is obvious. But the leaves corresponding to the slits are, as is well known, suppressed. And though De Bary² cites authorities for regarding the prickles of *Cactaceae* as 'emergences,' the view of Goebel³, that 'the spines are transformed leaves which arise upon very much reduced lateral shoots,' is no doubt correct.

Fig. 1 shows the short external branches of the *Loranthus*: they are a little longer than the spines, and perhaps derive some protection from them.

In Fig. 2 the haustorium of the *Loranthus* is seen to consist of a very irregular mass freely ramifying and sometimes anastomosing in the cortex of the *Cereus*. The surface of this mass consists everywhere of a uniform tough and indurated tissue, yellow when fresh but pale brown when dried. The dissection at once furnished the clue to a very enigmatical object of unknown history which I found amongst the *Cactaceae* in the Kew Museum. It is a hollow body with a thin wall of an irregular shape branched in all directions, and gives the impression of having been blown out from some plastic material which has afterwards set hard. The greatest diameter of the hollow portion is some two inches, and the

¹ See De Bary, 'Comparative Anatomy of the Phanerogams and Ferns,' p. 310.

² Loc. cit., p. 66.

³ Goebel, 'Organography of Plants,' pp. 168, 169.

greatest width of the whole specimen some five. One cannot but wonder, if such an object occurred in the fossil state, what the palaeo-botanist would make of it. That it at any rate represents a haustorium of *Loranthus aphyllus*, there can, I think, be no doubt.

The whole of this singular structure must be regarded as a modified root. It differs little from the similar structure luminously described by Sachs¹ in the Mistletoe (*Viscum album*), but it has been modified so as to adapt it to the peculiar nature of its host. The haustorium adheres here and there to the fibro-vascular cylinder, but I am disposed to think there is no real coalescence of the tissues: the condition of the material did not allow, however, of this point being definitely ascertained. As in *Viscum* the haustorium gives rise to shoots which break through the cortex and appear externally. The points marked *a*, *b*, *c*, and *d*, in Fig. 2, show where the shoots have disarticulated from the haustorium. They are destitute of chlorophyll: the plant is therefore wholly parasitic.

It is interesting to note that the aerial shoots always emerge, according to observers on the spot, on the upper side of the spine-tufts². I quote the following account of this from a letter received in 1894 from Mr. J. W. Warburton, at that time Consul-General at Valparaiso.

‘Another Quintral [the local name for *Loranthus*] grows on the tall Cactus. I found this at from 3,000 to 5,000 feet elevation. I had not seen any of it at low elevation near the coast, though the same Cactus is plentiful here.

‘This Quintral is very plentiful; I suppose two out of five Cacti, certainly one in five carrying it. On some it was in great masses and looked like bunches; the berries or fruit mostly red, ripening pink.

‘I examined some thousands of plants on at least a hundred or more Cacti, and I noticed one circumstance that struck me

¹ Lectures on the Physiology of Plants, pp. 25, 26.

² See Hemsley, Journ. Linn. Soc. Bot., vol. xxxi, p. 306, in which, however, the point is not quite clear.

as remarkable. Every plant without exception was rooted at the point from where the groups of thorns of the Cactus grow, and on the *upper* side of that point. I was unable to find one single instance where the Quintral sprang from either the furrow in the Cactus, or from the *lower* or lateral side of the groups of thorns.'

I was curious to ascertain the nature of the external coat of the haustorium. This is indeed all that represents it, as the internal tissues, which must have been soft, have all but completely decayed, leaving a mere inflated and hollow shell; and the investigation offered little promise of yielding any result of interest. I am greatly indebted to Mr. L. A. Boodle, F.L.S., for kindly making the attempt, with results as interesting as they were unexpected. He has kindly permitted me to add his observations in the following note.

Histology of the Haustorium.

BY L. A. BOODLE, F.L.S.

The branched body, of thalloid form, which was immersed in the cortical tissues of the *Cereus*, and which is seen in Fig. 2, might be thought at first to consist of nothing but the haustorial apparatus of the *Loranthus*. Microscopic examination, however, showed that its more external tissues were composed entirely of a periderm, whose characters proved it to belong to the host-plant.

The periderm encloses a central core of tissue, which must consist of a haustorium of the *Loranthus*, together with a certain amount of injured cortical tissue belonging to the Cactus; but unfortunately, in most places, everything within the periderm was found to have become withered or disorganized into a brown or nearly black mass, in which structure could not be recognized. Fig. 3 is a photograph of a transverse section of one of the small branches of the specimen. The periderm is clearest on the upper and lower sides in the figure, and is seen to consist of two kinds of tissue, thick- and thin-walled cells forming separate zones; *a.* and *c.* are two bands of the thick-

walled cells, and *b.* is the intermediate thin-walled layer, besides which remnants of a second thin-walled layer are seen on the outside. On the lower side in the photograph the periderm is thinner, and includes only one zone of each of the two kinds of cells. In *a.* and *b.* the radial arrangement of the component cells is clearly seen, but in the thin-walled tissue a general crumpling of the cells has taken place to such an extent, that their original radial arrangement is quite obscured. The part of this layer marked *b.* shows most indication of radial seriation. Comparison of different sections, however, showed clearly that the whole of the periderm must have been formed by the same phellogen, for, wherever the periderm was least crushed, the same radial rows were seen to be continued through all its layers. Fig. 4 shows a small piece of periderm, from a section similar to Fig. 3, more highly magnified. The radial arrangement is clear, as the thin-walled cells are not much crumpled. These cells are nearly colourless, and suberized, while the thick-walled cells have yellow walls, which, perhaps with the exception of the middle lamella, are lignified through their entire thickness. The walls show conspicuous stratification, and are provided with numerous pits; the latter, however, are not represented in the drawing.

The above characters agree well with the descriptions and drawings of cactaceous periderm given by Schleiden¹ and Arloing². The tissue in question, when compared with the wound-periderm and normal periderm of a species of *Cereus* grown at Kew, was found to be practically identical with both in structure.

The periderm is obviously a wound-periderm, which has been formed in the cortical tissues of the *Cereus*, so as to form a complete sheath enclosing the haustoria together with the adjacent injured cortical cells of the host-plant. The periderm was formed *towards* the haustorium, that is to say, the phellogen

¹ Schleiden, Beitr. z. Anat. d. Cacteen, Mém. de l'Acad. Imp. des Sci. de St.-Pétersbourg, 6^e sér., tom. iv, 1839, p. 18, and Pl. IX, Fig. 5 (*Echinocactus*).

² Arloing, Bouturage des Cactées, Ann. des Sci. Nat., Bot., 6^e sér., tom. iv, 1876, p. 5, and Pl. I, Fig. 2 (*Cereus*), &c.

would be found in a position peripheral to the whole tube of periderm. In the fresh specimen examined for periderm, the convexity of individual thick-walled cells was directed away from the phellogen; and as, in Fig. 4, *e. w.* is the external surface of a branch and the convexity of the thick cells is turned away from this, the phellogen must have been at the surface. The same may be proved by Fig. 3. The external walls (*e. w.*, Fig. 4) differed from the other thin walls of the same layer in being brownish, and in having similarly coloured granules adherent to them. The cells to which these walls belong are probably therefore *young* cork-cells, and the actual phellogen has been removed with the cortical tissues.

The sheathing of internal tissues by a periderm is of course a familiar occurrence in other plants, e.g. in the case of parts of potato-tubers, when affected by a kind of dry-rot as described by Bretfeld¹. Similar formation of periderm was observed by the same author (loc. cit.) enclosing internal tissues injured by purely mechanical means in *Begonia* and *Coleus*. By means of careful torsion of the stem, some of the internal parenchymatous tissues were ruptured without external injury to the stem, and after eighteen days the mass of injured cells was found to be completely enclosed by periderm.

For comparison with the periderm-formation around the *Loranthus*-haustorium, an interesting fact, described by Arloing and illustrated by him², should be mentioned, namely, that the adventitious roots of *Cereus monstrosus* (which branch on their way out through the cortex of the stem) are sheathed by a wound-periderm, formed by the cortical tissues of the stem, in precisely the same manner as has been described in the case of *Loranthus*-haustoria. The root also has periderm of its own.

To return to the specimen under consideration, it has already been mentioned that in most places all the tissues lying within the periderm had become devoid of recognizable

¹ Bretfeld, Ueber Vernarbung u. Blattfall. Jahrb. f. wiss. Bot., Bd. xii, 1879-81, p. 138.

² Arloing, loc. cit., Fig. 8, Pl. 2.

structure. In some branches, however, instead of the uniform brown mass seen in Fig. 3, a band of tissue immediately within the periderm was made out to consist of collapsed, brown-walled, rather large cells—no doubt withered cortical cells of the *Cereus*—while the more central part appeared to represent a different kind of tissue, more thoroughly disorganized. Near the tip of one branch examined, the condition was better, as shown in Fig. 5. Below the periderm (*p.*) is a zone of brown collapsed cells (*b. t.*), probably cortex of the *Cereus*, and the central region is filled with a colourless tissue (*c. t.*, shaded in the drawing). Parenchymatous cells were not clearly recognizable, but the tissue appeared to consist of collapsed cells, and gave a cellulose-reaction with Schulze's solution. Numerous small vascular bundles or strands of tracheides are present here (*v. b.*). They are quite irregularly placed, and composed chiefly of spiral elements. One may assume that the central pale tissue, containing the vascular bundles, is a haustorium of the *Loranthus*. Nothing further can be said about its structure except that one of the vascular bundles, which was rather larger than the rest (*a.* in Fig. 5) had an arrangement of its elements suggesting a collateral bundle. It is shown more highly magnified in Fig. 6. The rows of cells (*ph.*) are not collapsed, but have dense contents. They are probably phloem-parenchyma.

At the tips of branches examined there was no gap in the periderm. While the haustorium was growing in length, of course its apical region must have been free. The closing in of the tip may have occurred after the death of the haustorium; or perhaps after a time the haustorium may have ceased to grow in length, and become completely enclosed while still alive. The cortical cells of the *Cereus*, shut in by the periderm might, in that case, have afforded nutriment to the haustorium for some little time.

There is a possibility that one or two branches of the specimen might contain roots of the *Cereus*, instead of, or in addition to, haustorial tissue; for Arloing¹ finds that several

¹ Arloing, loc. cit., p. 32.

causes may lead to the formation of adventitious roots in the Cactaceae, and states that adventitious roots are often seen starting from a point of the stem which has been the seat of a contusion. A haustorium forcing its way into the neighbourhood of the pericycle or cambium might have caused root formation.

It is evident from the facts contained in these notes that there is still a good deal to be cleared up in connexion with the life-history and anatomy of this interesting parasite. It would be an easy and pleasant problem for any botanist who found himself in Chili with sufficient leisure for the purpose.

I have only to add that I am indebted to Lady Thiselton-Dyer for the excellent and accurate drawings of the naked-eye anatomy, and to Mr. Boodle for those of the microscopic details.

EXPLANATION OF FIGURES IN PLATE XL.

Illustrating Sir W. T. Thiselton-Dyer's Morphological Notes. IV.

Fig. 1. Portion of the external surface of a stem of *Cereus Quisco*, showing the aërial shoots of *Loranthus aphyllus*. It is possible that this may have been represented upside down.

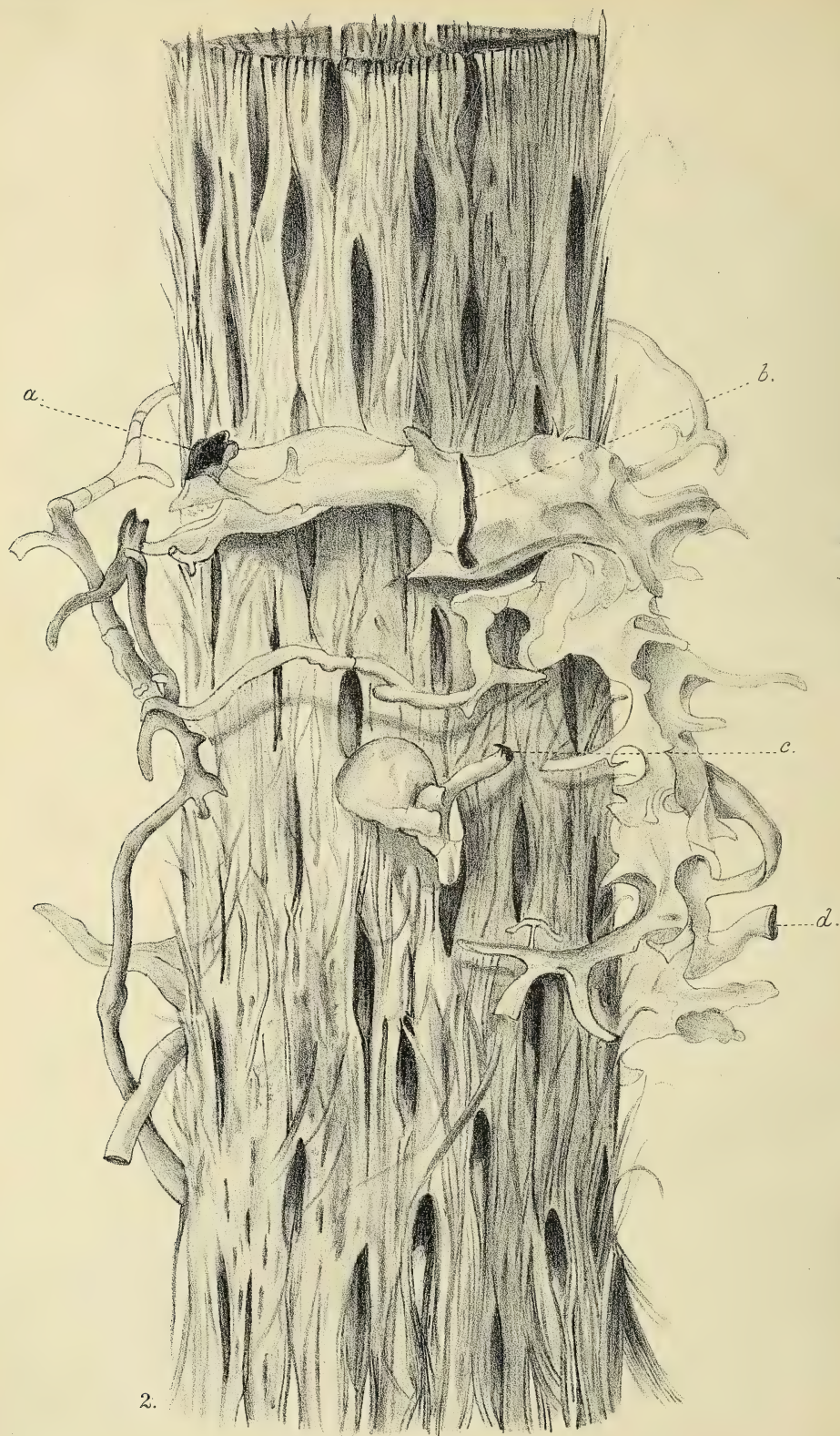
Fig. 2. Fibro-vascular cylinder of *Cereus Quisco* from which the cortical tissues have been dissected away leaving the haustorium (thalloid body) of *Loranthus aphyllus* in situ.

Fig. 3. Photograph of a transverse section of one of the smaller branches of the thalloid body. *a.* and *c.*, two zones of sclerotic periderm; *b.* zone of thin-walled suberized periderm. The periderm of the *Cereus* encloses brown disorganized tissues. $\times 40$.

Fig. 4. A piece of periderm from a similar section. The outer walls (*e. w.*) formed the external surface of the specimen. *r. w.*, crumpled radial walls. $\times 180$.

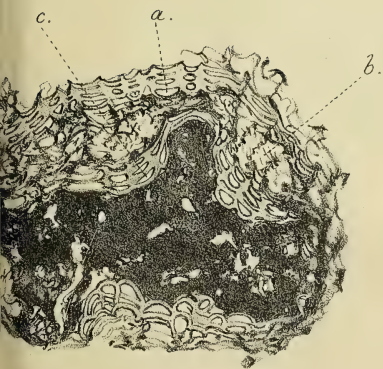
Fig. 5. Section close to the tip of a branch. *p.*, periderm; *b. t.*, brown crushed tissue; *c. t.*, central pale crushed tissue; *v. b.*, a vascular bundle cut longitudinally; *a.*, a vascular bundle cut transversely. $\times 45$.

Fig. 6. Enlarged drawing of the vascular bundle *a.* in Fig. 5. *ph.*, probably phloem-elements; *c. t.*, crushed tissue surrounding the bundle. $\times 390$.

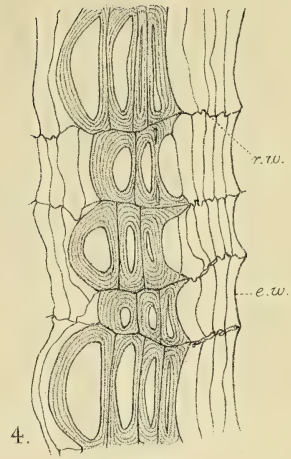




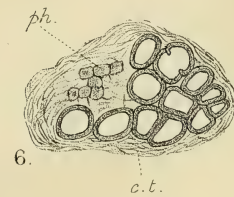
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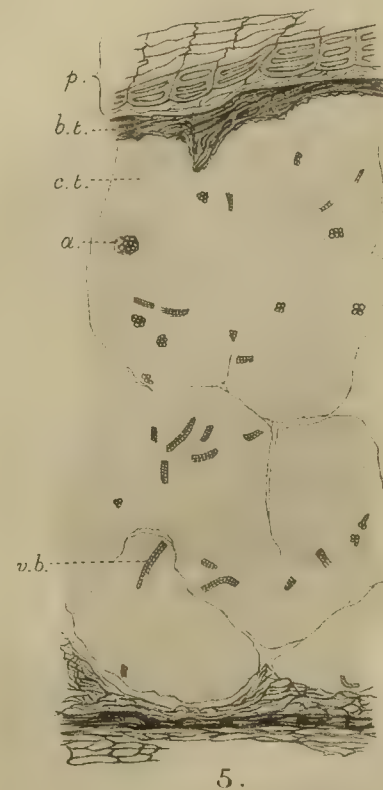
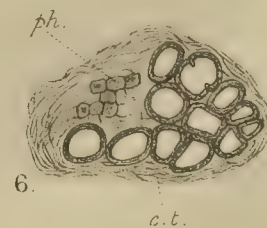
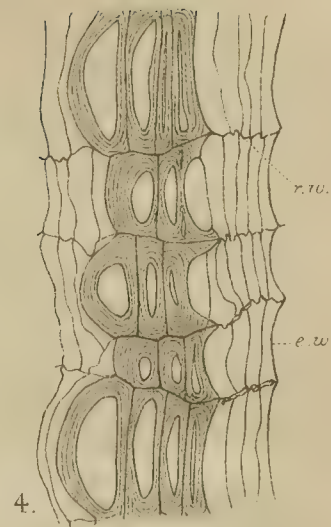
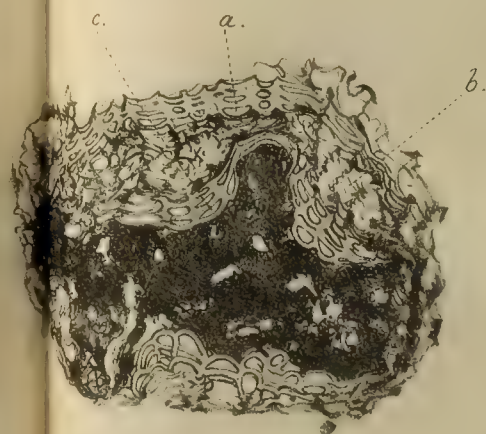
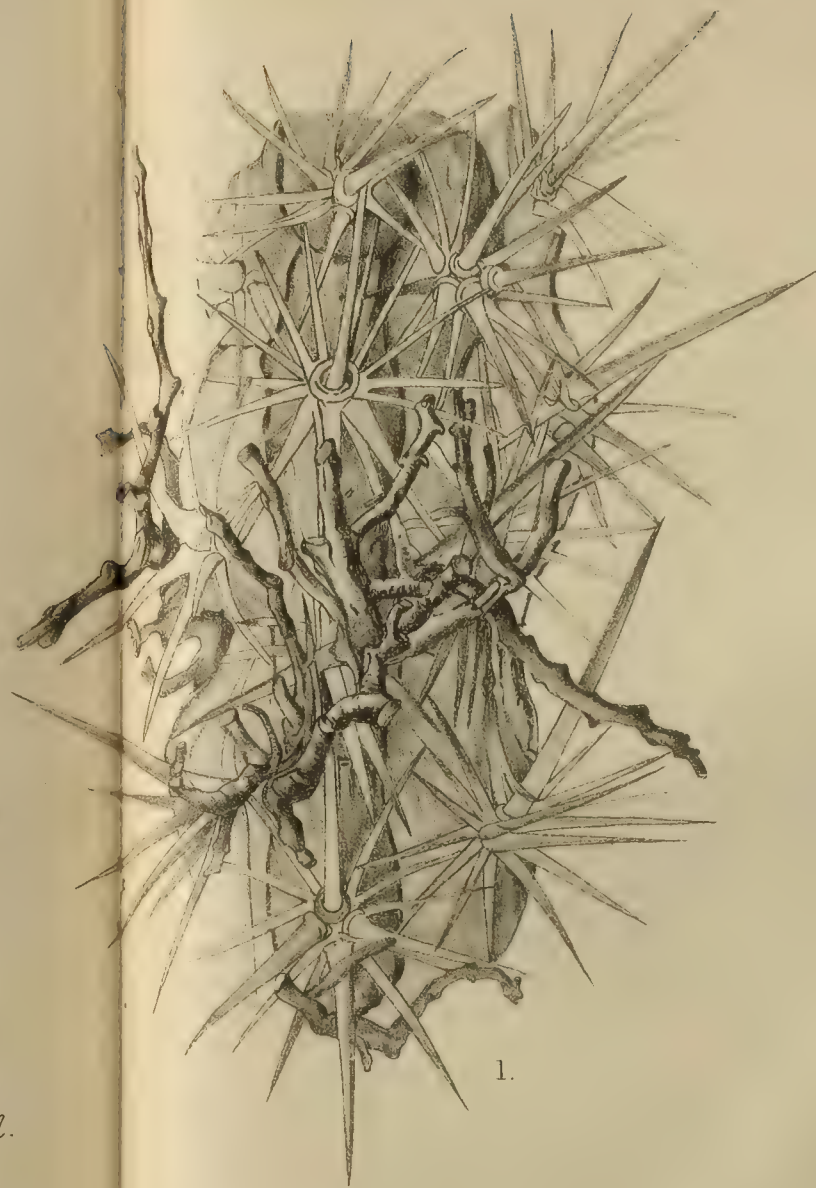
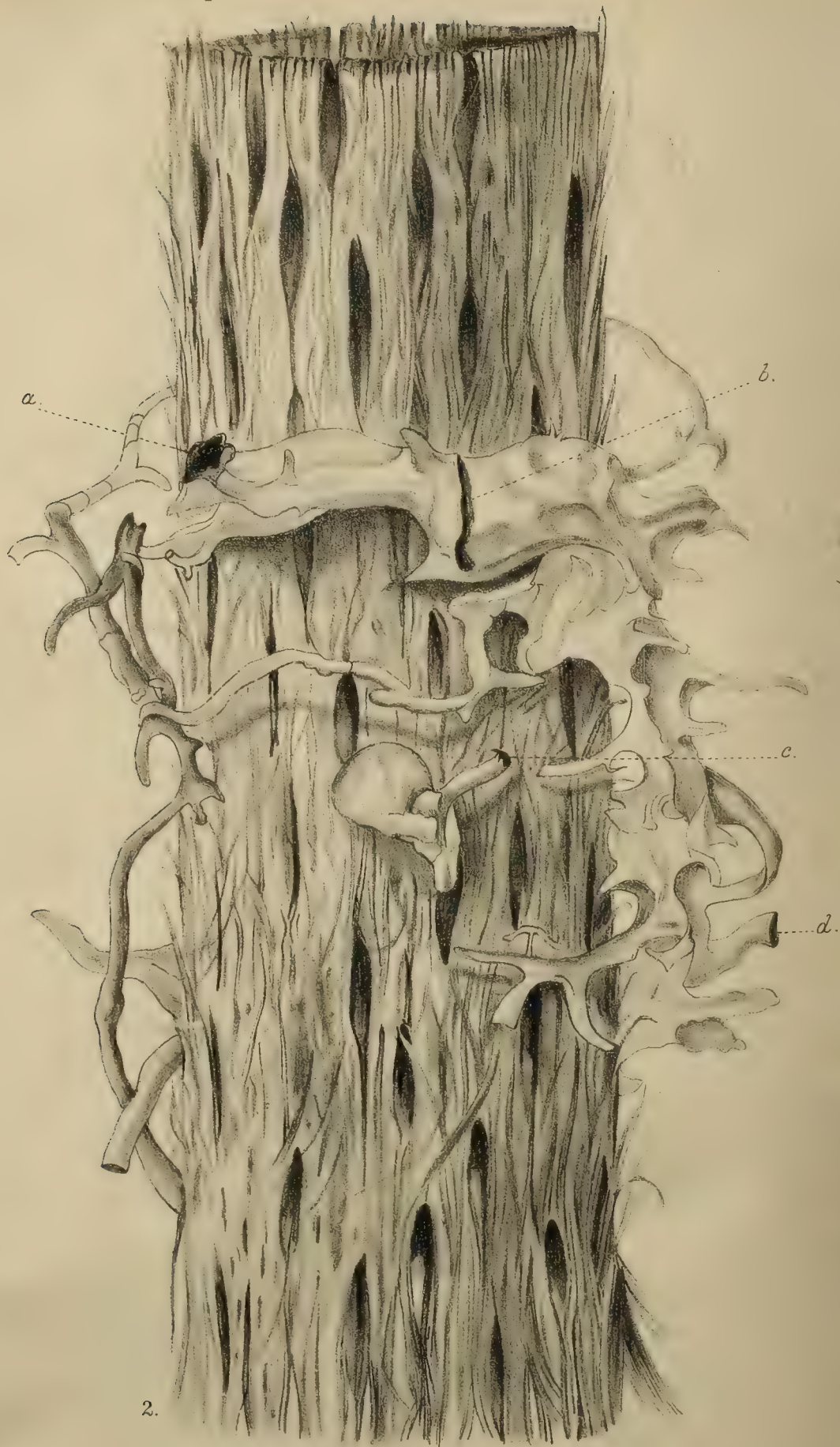
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NOTES.

SEXUAL SPORE-FORMATION AMONG THE SACCHAROMYCETES¹.—In a paper read before the Royal Society on June 6 of this year the author has described a Yeast-form, the vegetative propagation of which takes place by budding in a manner exactly similar to that of *Saccharomyces cerevisiae* (Hans.) and other budding Yeasts, but which is distinguished from them by a curious process of spore-formation. The usual process among the Saccharomycetes is apparently a very simple one, the spores being formed under appropriate conditions in any cell by the division of its contents into a number of bodies, each of which develops into a spore. In this particular case, however, spore-formation is preceded by a kind of conjugation between two cells. Considering the case of any pair of cells which are about to take part in the process, the first stage consists in the development by each cell of a small protuberance. These protuberances are developed from neighbouring parts of the two cells, and grow towards each other, evidently under the influence of some stimulus acting between them. Growth continues until their tips are in contact, and then a solution of the walls at that point occurs, so that a continuous canal of communication is opened between the cells through the bridge or neck formed by the junction and fusion of the protuberances. Spore-formation then takes place, usually in each of the cells, although in some cases spores appear in one of the cells only, the contents of the other disappearing and leaving merely an empty sac. The spores make their appearance in a manner similar to that occurring in the other Saccharomycetes. No cases have been observed in which spore-formation was not preceded by conjugation. The behaviour of the nuclei during the

¹ Read before the Botanical Section of the British Association, at the Glasgow Meeting, September, 1901.

process was studied in specimens hardened and stained at different stages. In all independent cells, before the protuberances are developed, there was found a deeply stained body placed more or less in the centre of the cell. This moved to the side of the cell to the point where the protuberance was put out, as soon as the development of the latter began. It took up a position at the tip of this, and remained there during the growth, until contact with the corresponding protuberance of the other cell taking part in the conjugation was reached. At the tip of this also a similar deeply stained body was situated, and on the fusion of the tips of the protuberances the two deeply stained bodies also fused, forming one large body filling the greater part of the junction between the two cells. This body subsequently divided into two portions, which were withdrawn from the neck into the bodies of the cells, and there again they underwent, as a rule, another division, each of the bodies so produced becoming the basis of a spore. In spite of the controversy as to the nature of the Yeast nucleus, there can scarcely be any doubt that the deeply stained bodies represent, in part at any rate, the nucleus, that consequently a nuclear fusion actually does take place, and that the whole process is a kind of isogamous sexual act.

It was pointed out in the paper that in the case of spore-formation in the fission-yeast, *Schizosaccharomyces octosporus* (Beijerinck), a process, somewhat approaching this in nature, was already known to occur. Schiönning ('95) described the details as follows:—Across the middle of a cell a partition-wall was formed: this splits, and the two new cells so formed assumed a round shape, remaining attached at one point. They then again coalesce, and form an hour-glass shaped cell, which increases in size and swells up, until it becomes of an oval shape. Spores, usually eight in number, are then formed within this. He gave, however, no account of the nuclear behaviour. Hoffmeister ('00), in a paper on the nucleus of Yeasts, gave figures showing that nuclear fusion took place, when the two cells coalesced, before the subsequent divisions of spore-formation occurred. Hence this process also must be regarded as a sexual act.

Since the publication of the above, Guilliermond has described a third case of a sexual act preceding spore-formation among the Saccharomycetes. He has not only verified and amplified the results of Schiönning and Hoffmeister for *Schizosaccharomyces octosporus*, but has also observed a somewhat similar process in *Schizosaccharo-*

myces Pombe. The details of the process in the latter case seem to be identical with those just described for *Zygosaccharomyces*. A pair of cells situated near together put out protuberances, which meet at their tips and fuse, spore-formation then following in the cells. The fused compound-cell usually retains the form of two cells united by a neck. In *Schizosaccharomyces octosporus* he finds that the method of fusion described by Schiönnig is not the most common. Usually a cell divides into two daughter-cells by a transverse wall. A small protuberance is formed at the extremity of each of these daughter-cells. These protuberances fuse and form a canal of communication. The separating wall disappears and the canal enlarges, the single cell so formed becoming gradually oval, and spores are rapidly formed within. Very often the fusion of the cells is not complete, while in exceptional cases the cells become converted into asci directly without any fusion taking place.

Since no mention is made of Hoffmeister's work nor of the case of conjugation in *Zygosaccharomyces*, Guilliermond has probably not yet seen the papers on those subjects.

He has gone into the question of the behaviour of the nuclei very fully, and has published earlier this year two papers, which contain the results of his work on the Yeast nucleus, and which must therefore be considered before giving details of the nuclear actions which he observed during the process of conjugation. He found in *Saccharomyces cerevisiae* and other species a nucleus of definite structure, consisting of nucleoplasm surrounded by a membrane and containing several granules, of which one, larger and more regular, is the nucleolus. The 'nuclear vacuole with chromatin granules' of Wager is, according to him, not nuclear in nature. He has studied the structure and development of these granules and the vacuoles in connexion with them in numerous species of *Saccharomyces* and particularly in a *Dematium*, sp. The granules are not fatty in nature, nor apparently are they proteid, since they resist the action of pepsin. Nuclein solvents leave them untouched. They are, however, likely to be overlooked after such treatment, since their staining is rendered more difficult. They possess the characteristics of the 'red grains' of Bütschli. They are usually situated in the interior of vacuoles, and have their origin in connexion with that of the latter. At their first appearance they are very small, and are found in small hyaline spaces, probably the precursors of the vacuoles. These increase in size, and

fuse together to form a large vacuole or vacuoles, while the granules also increase in size by fusion. The fine granules of Wager, which are contained in vacuoles, and which the latter author considered as nuclear, are shown to be identical in nature with the larger granules, which he considered as proteid. Dealing more particularly with the spore-formation in *S. Ludwigii*, a small nucleus with no observable structure is found in young vigorous cells in connexion with a vacuole containing numerous small granules, the 'red grains' of Bütschli. Later, one or two more vacuoles containing glycogen appear at the poles of the cell. In the first stages of sporulation each of these divides, until the protoplasm appears to be entirely filled with small vacuoles separated by very fine meshes of protoplasm. The glycogen vacuoles and the vacuoles containing 'red grains' can be distinguished. Later the vacuoles containing 'red grains' change in appearance and the granules diminish in number and size, the vacuoles themselves then staining a uniform pale-red colour with those stains which give the 'red grains' their characteristic red tint (haematoxylin, gentian violet, &c.). This colouration of the vacuoles is explained by the solution of the granules, since it never occurs at other times. At this stage the nucleus divides into two daughter-nuclei, which travel to either end of the cell and divide again. The division is intermediate between direct division and karyokinesis. Each of these nuclei becomes the basis of a spore, which is formed by the condensation of the protoplasm around a nucleus. The epiplasm contains a substance that stains uniformly red, when the spores are very young, but this gradually disappears as the spores ripen. On account of these facts Guilliermond regards the 'red grains' as reserve material, which is used up in spore-formation.

Returning now to his account of the nuclear behaviour of *S. octosporus* during spore-formation, he states that the nucleus of this form consists of a deeply staining nucleolus surrounded by non-staining nucleoplasm with traces of a limiting membrane. It is in the neighbourhood of small vacuoles or one large vacuole containing 'red grains.' The nuclei in the cells about to conjugate are situated close to the point of fusion. When the wall disappears the two nuclei fuse. The fused nucleus soon divides into two daughter-nuclei, which travel into the cells and take part in spore-formation divisions. The 'red grains' disappear at the moment of fusion. Similar phenomena are observed in *S. Pombe*.

There is thus in these two cases an exact parallel to the behaviour of *Zygosaccharomyces* during spore-formation, and there can be very little doubt that a sexual act takes place here, as in the case already described by the author of the present communication. Having regard to the irregular forms of spore-containing cells of such species as *Schizosaccharomyces mellacei* and *S. Comesii*, which closely resemble the spore-containing cells of *S. Pombe* and *Zygosaccharomyces*, it seems very probable that in these cases also conjugation takes place as a preliminary to spore-formation.

In the three cases of conjugation which have now been observed, there seem to be suggestive modifications of the process. In *Zygosaccharomyces* the ultimate shape of the compound cell remains the same as it is found immediately after conjugation; i. e. two more or less ovoid cells joined together into one compound cell by a conspicuous neck or bridge, which under certain conditions can be made to grow out to some considerable length. In *S. Pombe*, while the compound cell has a similar form to that in *Zygosaccharomyces*, in some cases it loses this structure, being modified so as to appear as a rather enlarged ovoid cell. In *S. octosporus* the latter is the usual occurrence, all traces of the two individual cells being lost. Moreover, in this species conjugation does not always seem to occur, a single cell becoming an ascus without any conjugation with another cell. In the light of these facts, Janssen and Leblanc's observation on the division of the nucleus of the spore mother-cell into two nuclei, and the subsequent fusion of these previous to the series of divisions which yield the nuclei of the spores, becomes very interesting. If Guilliermond's statement, that Wager's 'chromatin-containing vacuole' is not a part of the nuclear apparatus, be correct, then the latter's interpretation of the fusion described by Janssen and Leblanc cannot suffice. It is true that Guilliermond has not observed such fusions, but he evidently does not consider that that fact proves that they do not occur, for he proposes to investigate the point specially.

A confirmation of Janssen's and Leblanc's observations would throw considerable light on the question of spore-formation among the *Saccharomycetes*, strongly supporting the hypothesis that it is a very much reduced sexual act. The gradual reduction or degradation of this act is traceable through the forms described above. In *Zygosaccharomyces*, independent cells fuse, retaining their individuality after fusion. In *S. Pombe* this is also usually the case, but

is less pronounced. In *S. octosporus* two cells, the products of a single mother-cell, fuse, usually completely, so as to reproduce practically the mother-cell, spore-formation then occurring. In occasional cases, however, the mother-cell does not divide into two daughter-cells which fuse again, but proceeds at once to the formation of spores. Such cases would represent a still further suppression of the individuality of the conjugating cells, if it be shown that a nuclear division, followed by re-fusion of the two daughter-nuclei, does occur previous to spore-formation, the two nuclei representing the two daughter-cells of the usual process. If no such process takes place, it may be regarded as the extreme case of reduction of the sexual act, the spores being produced parthenogenetically or, as it appears, asexually.

There is, then, a fair amount of evidence to support the view that the *Saccharomyces* are derived from Fungi, which possessed a complete sexual act, and that this act has suffered in most cases so great a reduction as to be represented merely by the fusion of the two nuclei in the spore mother-cell, if Janssen's and Leblanc's observations be correct, or for all traces of it to have disappeared, if these authors be wrong.

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B. T. P. BARKER.

CAMBRIDGE.

ON AN ANOMALOUS LEAF OF *ANEMIA HIRSUTA*, SW.

—Abnormal leaves in the genus *Anemia* have been described by Prantl¹. One of the cases he mentions is that of a plant of *A. Phyllitidis*, Sw., in which the lowest primary segments of the leaf were quite or partly sterile, but were branched in the manner characteristic of the fertile pinnae.

A somewhat similar anomaly occurring in a leaf of *A. hirsuta*, Sw., seems worth mentioning on account of the conditions under which it was produced. In this case the lowest pair of pinnae, which would be the fertile ones in a fertile leaf, are quite sterile, but resemble fertile pinnae in that they are stalked and stand up vertically on the ventral side of the rachis. The laminae of these two pinnae are just as in the sterile ones, but are seated on stalks half an inch long, while sterile pinnae are typically sessile in this species, and the fertile pinnae of a normal fertile leaf produced by the same plant have stalks three inches in length. The plant in question was grown from one of several rhizomes kindly sent to me last summer by Mr. W. Fawcett, F.L.S., Director of Public Gardens and Plantations, Jamaica. The expanded leaves had been cut off leaving an inch or so of petiole, and the rhizomes were packed so as to keep damp for some time. By the time they arrived in England, however, the rhizomes and roots were quite dry externally. As the plants were intended for microscopic examination, only two of them were set apart for the purpose of trying to induce them to grow. This was successfully done in the Royal Botanic Gardens, Kew. One of the plants has produced several rather small sterile leaves, while the other plant put up first the abnormal leaf described above, and then a normal fertile leaf. Considering the facts of the case, it seems probable that the rudiment of a fertile leaf had been formed under normal conditions, but that the unfavourable conditions of transit, &c. caused it to complete its development in the manner characteristic of a purely vegetative leaf, as far as was still open to it. Expressed generally this may perhaps be said to be due to a check to the vitality of the plant, but the determining cause may well have been the lack of sufficient food-material of the kind required for the development of sori. It is probable that in a case like this, where the fertile pinnae are destitute

¹ Prantl, Unters. z. Morph. d. Gefässkryptogamen; II. Schizaeaceen. 1881, p. 20.

of a laminar expansion, the food-materials for sporangial development would not be manufactured locally, but brought from a distance. As the vascular bundles of the fertile pinnae are often decurrent in the petiole for some distance, there are grounds for supposing the reserves of the stem to be the source of the requisite substances. In the case under consideration these substances may have been present in the stem when the rudiment of the leaf was formed, but gradually destroyed in connexion with respiratory processes while the plant remained packed up and shorn of its leaves. Or perhaps only the supply of these materials to the developing leaf was prevented by the previous drying of some of the conducting tissues in the stem.

There is no need to try to explain the production of nothing but normal sterile leaves by the other rhizome. It may have previously produced no rudiments of fertile leaves, or none sufficiently far advanced to determine the form of the pinnae.

It should be pointed out that, although the production of an anomalous leaf immediately after the plant had been subjected to very unusual conditions may be a mere coincidence, a series of cultural experiments would probably decide the point, and might lead to very interesting results.

L. A. BOODLE.

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THE VASCULAR STRUCTURE OF THE 'FLOWERS' OF THE GNÉTACEAE:—With a view to determining how much nearer, if at all, the vascular structure of the 'flowers' of the Gnetaceae approaches to the more primitive type of the Cycadaceae than do the vegetative parts of these plants, I made a careful examination of the axes and foliar members of the flowers of all three genera, with the following results.

The bracts (both those of the general inflorescence and individual flowers) exhibit practically the vascular structure of the foliage leaves of the same plant, viz. a collateral bundle with transfusion-tissue fairly well developed occurring in a lateral position. In the bract of the female inflorescence of *Ephedra distachya*, however, I distinctly observed a few tracheides of *centripetal xylem* on the ventral side of the bundle, and, as I have noted in other plants, there occurs more immediately in the *median* ventral position a second group of proto-xylem, although very slight in amount, belonging to the centripetal

xylem. Hence, there is found in the foliar organs of these female inflorescences what I regard as a relic of the more primitive structure of the older types of Coniferae and of the Cycads, and which is not found in the vegetative foliar organs of the plants concerned.

The male sporophylls (on the view which I prefer taking of them) of the three genera are constituted according to the *radially-symmetrical* type. In *Ephedra* they are grouped intimately together so as to form a single columnar structure bearing the pollen-sacs at the apex. In *Welwitschia* they are fused, at least at the base, three and three together, into two groups on opposite sides of the flower. In *Gnetum* two sporophylls are intimately united together to form, as in *Ephedra*, a single column. In accordance with the radial or cylindrical structure the vascular bundle of the sporophyll in all three genera consists of what I must regard as a reduced *concentric* structure of which only the few small, central, spiral tracheides, forming a circular group, remain, the phloëm being absent or quite undistinguishable from the surrounding parenchymatous tissue. In this structure of the sporophyll we find a primitive type, which has been lost in Coniferae and modern Cycads, but which occurs in the fossil genus *Bennettites*, as also in *Ginkgo*¹. It is remarkable that this ancient type of sporophyll, with its corresponding vascular structure, should have been preserved in the otherwise advanced group of the Gnetaceae.

In the peduncle and axis of the female 'cone' of *Welwitschia* a most interesting structure obtains. The bundles of the innermost ring or central cylinder, twenty-five or so in number, frequently have an *inverted* strand attached to their dorsal side; the same phenomenon precisely which I have observed in the peduncle of some Cycadean cones²; this inverted strand may sometimes be so fused laterally with the main strand as to form a concentric structure of which the phloëm occupies the centre. But the most remarkable part of the whole structure consists in the presence of a number (about twelve) of rather widely-separated strands which belong for the most part to the *concentric* type of structure, although in reality only some two or three, in any one transverse section, possess this structure in perfect form, the others being more or less curved or horse-shoe-shaped; or, again,

¹ Worsdell, 'The Affinities of the Mesozoic Fossil *Bennettites Gibsonianus* Carr.' Ann. Bot., December, 1900.

² The Vascular Structure of the Sporophylls of the Cycadaceae. Ann. Bot., vol. xii, 1898, Plate XVII, Figs. 2 and 4.

two or three bundles are approximated together with their xylems mutually directed towards each other, to produce what is really the same result, viz. a *concentric* strand; in fact, all the strands are variations of one and the same type of structure, viz. the *concentric*. The bundles mostly exhibit, like those of the central cylinder, an *inverted* portion on their dorsal side, and this latter in its turn may have a small normally-orientated strand in close proximity to its outer face. Bundles, usually of collateral structure, are seen here and there between the two rings: these are the connecting-strands between the central cylinder and the outer concentric bundles¹.

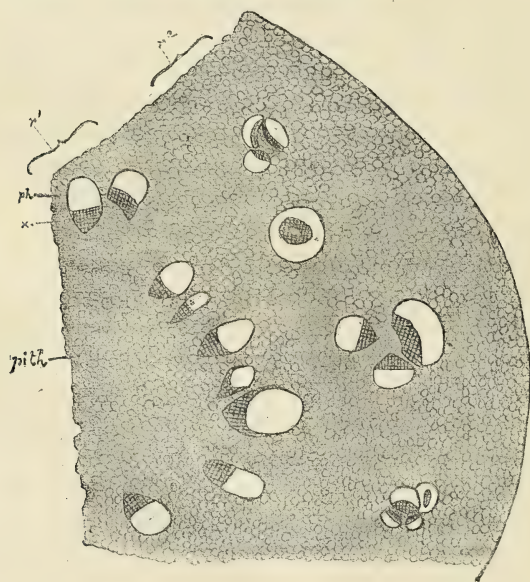


Fig. 10. *Welwitschia mirabilis*, Hk.—Part of transverse section of the stalk of the female inflorescence, showing the concentric strands of the second vascular cylinder, r^2 (Diagrammatic). r^1 , central cylinder. x , xylem, ph , phloem.

Here and there is a very small bundle, collateral in structure and with normal orientation, lying *within* the central cylinder in the pith.

Another striking part of the structure consists in the presence and enormous development of what I feel, although with some hesitation, inclined to regard as the representative of *centripetal xylem*; it occurs

¹ For similar strands connecting two cylinders see my paper: 'The Comparative Anatomy of certain species of *Encephalartos*,' Trans. Linn. Soc., vol. v, pt. 14, 1900, Fig. 2.

opposite the protoxylem of very many of the bundles of the central cylinder in the form of very small, lignified elements equalling in size the innermost primary tracheides of the bundles. In many places these small elements are seen to pass gradually over into larger and larger lignified cells which, in transverse section, are seen to be scattered on all sides, around the bundle and far out into the pith, as reticulate elements of the same shape as the adjacent parenchyma-cells. As seen in longitudinal section, the smallest of these presumably centripetal tracheides, viz. those nearest the protoxylem of the cylinder, are very faintly and indistinctly outlined as compared with the tracheides farther out in the pith, which fact points to their representing a tissue on the way towards becoming effete. It is true all these tracheides bear but little resemblance to the centripetal xylem known to us in other plants, for they possess *simple* pits, and thus in appearance suggest the idea of ordinary sclerotic cells; but the typical sclerotic cells of the plant occur side by side with them, and curious, slightly-lignified groups of fibres are also scattered throughout pith and cortex. Moreover, I can detect no difference between the character of the pitting of these elements and that of the secondary tracheides of the bundle itself; and further, the gradual transition existing between the much elongated, narrow elements adjoining the protoxylem of the cylinder, and the short, reticulate, parenchyma-like elements of the more distant region, is precisely the same phenomenon met with in the foliar bundles of Cycads and Coniferae which exhibit centripetal xylem, and is unlike anything we should expect to find in a system of sclerides. In fact, I conclude that we may have here, as in the similar instance of the stem of *Cephalotaxus* (*Podocarpus*) *koraiana* observed by Rothert¹, and the peduncle of *Stangeria*, &c.², a case in which the centripetal xylem of the bundles of the *axis*, instead of becoming, as in the great majority of cases, entirely suppressed and extinct, is retained either as a mechanical tissue or as a mechanical and conducting tissue combined; but the pitting of whose elements, in the case before us, has become modified, and which has become extended throughout the ground-tissue, as in the case of the centripetal xylem

¹ Rothert, W., 'Ueber parenchymatische Tracheiden und Harzgänge im Mark von *Cephalotaxus*-Arten.' Berichte d. deutsch. bot. Gesell., Bd. xvii, 1899.

² Scott, D. H., 'The Anatomical characters presented by the Peduncle of Cycadaceae.' Ann. Bot., vol. xi, 1897, Plate XX, Fig. 2, and Plate XXI, Figs. 8 and 9.

of Coniferae, a relic of an ancient, ancestral character. But I am still quite willing to admit that this view of the tissue concerned may be an erroneous one. The *quasi* centripetal xylem of the inverted strands permeates the cortex in the same way as does that of the central cylinder the pith.

But there is another interesting point exhibited by the primitive floral axis of *Welwitschia*. I recognize an ancestral type of structure in the presence of the concentric, or partially-concentric, strands outside the central cylinder. These strands are, however, as must necessarily follow from the fact that they eventually supply lateral axes, *primary* in origin; but I nevertheless regard them as homologous and comparable with the strands composing the second cylinder in the vegetative axes of four genera of Cycads and of *Welwitschia* itself, which are not primary but *secondary* in origin.

I do not myself consider it necessary, as regards the phylum of plants with which we are dealing, but, on the contrary, rather misleading, to lay much stress on the difference between primary and secondary structures; for the various cylinders, where they occur, all have, with the exception of the insignificant and often transitory protoxylem and protophloem of the former, exactly the same constitution and appearance. In fact, it seems to me that the *only* difference between them, and that an unimportant one, lies in the *varying periods* at which they are formed. *All* the cylinders, from the innermost primary one outward, must be homologous one with another. The phenomenon here exhibited by *Welwitschia* I believe, however, to be parallel with the case of *Medullosa Leuckarti*, Göpp. and Stenz.¹ in whose stem *two primary* cylinders have been described; with that of *Ceratozamia mexicana*, Brongn., and *C. latifolia*, Miq.² in the axes of whose male cones I have myself described what I believe to be the rudiment of an *intrafascicular* primary cylinder of bundles; and, finally, with that of *Encephalartos*³, the male and female cones of which exhibit in their axes *two* cylinders of *primary* bundles, the outermost of which supplies the sporophylls, and, in consequence, dwindles in the extent of its development towards the apex of the cone; in both *Welwitschia*

¹ Weber und Sterzel, 'Beiträge zur Kenntniss der Medulloseae,' p. 42, and Plate IV, Fig. 2, 1896.

² Worsdell, 'Contributions to the Comparative Anatomy of the Cycadaceae,' Trans. Linn. Soc., vol. vi, pt. 2, p. 117, and Plate XVI, Figs. 10 and 11, 1901.

³ Ibid., p. 116, and Plate XV, Figs. 7 and 9.

and *Encephalartos* the collaterally-constructed extrafascicular strands of the vegetative axis reappear in the more primitive axis of the cone as more or less concentrically-constructed strands. The explanation of the fact that the second cylinder of the cone is primary, while that of the vegetative axis is secondary in origin, may be found in the fact that in the former case the strands of the outer cylinder are formed quite early, and assume the function of supplying the sporophylls, whereas, in the latter case, it is the inner cylinder alone which, in the first instance, supplies the leaves, and which goes on developing in thickness for some time before the formation of a second cylinder is demanded, in order to assist in the general conductive function of the stem, and, like the subsequent cylinders, to take a part in supplying the leaves. The late period at which the second cylinder arises necessitates its earliest beginnings being inaugurated by a *cam-bium*; but this differing mode of origin cannot in the least affect the fact that this 'secondary' extrafascicular cylinder of the vegetative axis is directly homologous with the 'primary' extrafascicular cylinder of the peduncle of *Encephalartos*. Nevertheless, the possibility remains, which is not improbable, that this latter is equivalent to the innermost or primary cylinder of the vegetative stem of the same plant, and that the innermost cylinder of the peduncle represents an intrafascicular one, such as that existing in the cases above mentioned.

In *Welwitschia* and the other plants which exhibit it we may regard the concentric structure as having been retained in the second cylinder of strands, owing to the fact that there has been, in the region where they occur, less demand for a modification of the structure to suit the strengthening and other requirements of the plant, and it is just in those regions of the axis where, *a priori*, we should expect primitive ancestral characters to turn up that they actually do occur.

I am, of course, aware that the one or two extra rings of bundles found in the peduncle and axis of the cone, and which have been clearly figured by Strasburger¹ both for *Welwitschia* and *Gnetum* (to whose striking figure of the cone-axis of the former plant I would direct special attention), are of only local and thus transitory occurrence, for they are the strands supplying the flowers axillary to the bracts, and in the region of the axis above the uppermost flowers are

¹ Die Coniferen und Gnetaceen, Plate XX, Fig. 49 (*Welwitschia*); Plate XXI, Figs. 17-22, and 36-7 (*Gnetum*), 1872.

no longer present. For all that, I hold these transitory, incompact extrafascicular rings of bundles to be homologous with the more fixed and permanent extrafascicular rings of bundles in the vegetative axes both of this plant and of Cycads; the indefinite, strong, vegetative growth of the axes in the latter case being the cause of the greater fixity, compactness, and stronger development of the extrafascicular strands there occurring. The fact that the extrafascicular concentric strands of *Welwitschia* become all used up in supplying the flowers does not appear to me to affect the question of their homologies in the least; the collateral extrafascicular strands of the vegetative axes of Cycads are dependent for their existence on the presence of the foliage-leaves, and take a large part in supplying them with vascular tissue; and they are not present at the apex of the stem above the last incoming leaf-traces.

If the concentric strands composing the outer ring in the axis of the cone of *Welwitschia* do not represent a relic of the same ancient structure which prevails in the cauline organs of Cycads, what, I would ask, has induced the concentric structure of these strands to appear at all? why do we not find a ring of ordinary endarch strands such as compose the central cylinder? The arrangement and composition of these peculiar strands is far too irregular to admit of the possibility of their being governed solely by the structural symmetry or other characters of the floral axes which they supply; and, moreover, they do not pass obliquely outward to these axes as is the case with the steles supplying lateral branches in most axial organs, but form a constituent part of the vascular structure throughout the peduncle.

The small bundles occasionally found within the central cylinder may be the sole remnants of a third innermost cylinder of bundles.

In a peduncle of a female cone of *Gnetum* which I examined, essentially the same structure, as regards the presence of the medullary lignified tissue, is exhibited; but the second ring of strands is in this plant reduced to a small, incomplete concentric strand, or an arc-shaped row of strands, or a single endarch bundle lying, here and there, immediately outside one of the bundles of the central cylinder. The inverted strands are almost entirely absent.

The male cones present no striking features.

W. C. WORSDELL.

JODRELL LABORATORY, KEW,
October 10, 1901.

ON A PRIMITIVE TYPE OF STRUCTURE IN CALAMITES¹.—Palaeontological research has afforded evidence that the Horsetails and Lycopods—groups now so distinct—had a common origin. The class Sphenophyllales, restricted, so far as we know, to the Palaeozoic epoch, combines in an unmistakable manner the characters of Equisetales and Lycopodiales, while at the same time presenting peculiar features of its own. Broadly speaking, it is in the external morphology and in the reproductive structures that the Equisetales are approached, while the anatomy has an evidently Lycopodiaceous character.

The synthetic nature of the Sphenophyllales, indicated clearly enough in the type-genus *Sphenophyllum* itself, comes out still more obviously in the new genus *Cheirostrobos*. Here the general morphology of the strobilus, the form and structure of the sporangiophores and of the sporangia themselves, are all of a Calamarian type, while the anatomy of the axis is as clearly Lycopodiaceous in character.

So far, nothing has been found to bridge the gulf which separates the anatomy of the Calamariaeae (Palaeozoic Equisetales) from that of the Sphenophyllales or the Lycopods. The most ancient known genus of Calamariaeae—*Archaeocalamites*—approaches the Sphenophyllales in the superposition of the foliar whorls and in the dichotomous subdivision of the leaves, points on which Professor Potonié, especially, has laid stress. Anatomically, however, according to the researches of Dr. Renault and Count Solms-Laubach, it was an ordinary Calamite, differing in no essential respect from those of the Coal-measures. The stem of *Archaeocalamites*, like that of its later allies, had a large pith, surrounded by a ring of collateral vascular bundles, the wood of which, primary as well as secondary, was wholly *centrifugal* in development, the first-formed tracheides lying on the border of the pith, at the points marked by the carinal canals. In *Sphenophyllum*, on the other hand, the whole of the primary wood was *centripetally* developed, and there was no pith. In *Cheirostrobos* the same holds good, except that an insignificant portion of the primary wood may possibly have been added in a centrifugal direction. In Lycopods there may or may not be a pith, but the whole (*Lycopodium*, *Psilotum*,

¹ Abstract of paper read before the Botanical Section of the British Association, Glasgow, September, 1901.

Lepidodendron) or the greater part (*Tmesipteris*) of the primary wood is centripetal.

The Calamite which forms the subject of the present communication occurs in the well-known Burntisland beds of the Calciferous Sandstone Series, at the base of the Carboniferous Formation. The material is calcified, and the structure excellently preserved, though the specimens so far discovered are small and fragmentary. Their interest depends on the fact that each vascular bundle possesses a distinct arc of centripetal wood on the side towards the pith. The carinal canals are present, as in an ordinary Calamite, and contain, as usual, the remains of the disorganized protoxylem. They do not, however, as in other Equisetales, form the inner limit of the wood, but xylem of a considerable thickness, and consisting of typical tracheides, extends into the pith on the inner side of the canal, which is thus completely enclosed by the wood. Hence, starting from the spiral tracheides of the protoxylem, there was here a considerable development of xylem in a centripetal as well as in a centrifugal direction. That the organ was a stem, and not a root, is proved, not only by the presence of the carinal canals, but by the occurrence of nodes, at which the outgoing leaf-traces are clearly seen.

This appears to be the first case of centripetal wood observed in a Calamarian stem, and thus serves to furnish a new link between the Palaeozoic Equisetales and the Sphenophyllales, and through them with the Lycopods.

The specimens have not as yet supplied any evidence as to the superposition or alternation of the verticils, so we are not at present in a position to determine the genus to which they belonged. Provisionally, until further investigation has cleared up this question, the new stem may bear the name of *Calamites pettycurensis*, from the locality where it occurs.

D. H. SCOTT.

KEW.

REMARKS UPON THE NATURE OF THE STELE OF EUISETUM¹.—The vascular bundles of *Equisetum* are usually compared with those of a monostelic Phanerogam both in structural detail and with regard to their course out into the leaf. The following

¹ Abstract of paper read before the Botanical Section of the British Association, Glasgow, September, 1901.

observations made upon the stems of *E. Telmateja*, &c., show that this comparison cannot be satisfactorily maintained.

It was found that of the three strands of xylem present in each bundle of the internode, the carinal strand alone passes out at the node as a leaf-trace. The two lateral strands join on to the xylem of the nodal ring, and in certain species (*E. hiemale*, and better still in *E. giganteum*) they may be traced as externally projecting ridges over the nodal xylem into the internode above. In passing through the node they diverge from one another so that in the internode they are found on the adjacent sides of two different bundles. At the node above they approach each other, and in the next internode they both occur in the same bundle once again. The leaf-trace protoxylem, having entered the bundle, runs downwards for one internode between the two lateral strands; at the node below it divides into two branches, which curve to the right and the left in order to fuse with the neighbouring leaf-traces that enter at this node.

So the xylem of the so-called vascular bundle of *Equisetum* consists of three strands, two of which are lateral and cauline, while the median, or carinal, strand is common to both stem and leaf. The fact that only a small portion passes out as a leaf-trace, and not the bundle as a whole, constitutes an essential point of difference between it and the bundle of a Phanerogam.

The tracheides in each strand are very few, and consequently it is difficult to determine the direction of their development. However, as regards the leaf-trace and the carinal strand, it appears clear that they are not exarch but endarch, or perhaps slightly mesarch on the adaxial side. The lateral strands, as a whole, are differentiated later than the carinal strand (as might be expected from the close relation of the latter to the leaves), but they do not seem to be a continuation of its centrifugal development. On the contrary, in *E. giganteum*, where as many as ten to fifteen elements are present in each lateral strand, the smallest of them are invariably at the outer extremity, and they gradually increase in size inwards. Longitudinal sections show that the largest tracheides are coarsely reticulate, with large pits and very broad bands of thickening between them; in the smaller elements the reticulation becomes finer and more regular, and in the smallest it closely resembles true spiral thickening. To state definitely whether the lateral strands are exarch or not was not possible, because no incompletely differentiated portions of the stem were available; so the

question must remain at present undecided, although the mature structure certainly gives a strong impression of centripetal development. Potonié¹ has established a comparison between the secondary vascular tissues of the *Calamariaceae* and the *Sphenophyllaceae* by mentally doing away with the central mass of primary xylem that exists in the latter. By inverting this procedure, and considering it possible that the ancestors of the *Equisetums* may have possessed a xylem that extended to the centre of the stem, one is led to derive their structure, as it exists at present, from the modification of a stele with a solid central mass of centripetal xylem such as that of *Sphenophyllum*, or of certain *Lepidodendreae*. To illustrate the nature of the modifications that such a stele would have to undergo, a series of parallel developments may be pointed out within the latter group (*Lepidodendron Rhodumnense*, *selaginoides*, *Harcourtii*, *Sigillaria spinulosa*, and *Menardi*), in which parenchyma appears in the xylem, and gradually increases in quantity until only an attenuated peripheral ring of xylem remains, which then becomes more or less broken up into separate strands.

It is suggested that the lateral xylem-strands in the vascular bundles of the existing *Equisetums* may perhaps be taken to represent the last remnants of a primitive central mass, and that this would be entirely in agreement with their apparently centripetal development, and in particular with their cauline course.

D. T. GWYNNE-VAUGHAN.

GLASGOW.

SOME OBSERVATIONS UPON THE VASCULAR ANATOMY OF THE CYATHEACEAE².—In a number of *Dicksonias* with creeping or prostrate stems it is shown that the vascular system is solenostelic, the leaf-traces departing as a single strand curved into the form of a horseshoe, with its concavity facing towards the median line of the rhizome—*Dicksonia adiantoides*, *cicutaria*, *davallioides*, *apiifolia*, and *punctiloba*.

In *D. apiifolia* it is found that along the free margin of the leaf-gap there is a considerable increase in the amount of xylem in the

¹ Pflanzenpalaeontologie, p. 205.

² Abstract of paper read before the Botanical Section of the British Association, Glasgow, September, 1901.

solenostele, causing it to project somewhat towards the centre of the stem.

A similar marginal enlargement also occurs in *D. adiantoides*; and here it is continued past the leaf-gap, forming a ridge on the internal surface of the solenostele, running from one leaf-gap margin to another. In the internode this projecting portion of the xylem becomes separated off from the rest and surrounded by a phloem of its own; however, it remains always included within the same endodermis.

In *Dicksonia rubiginosa* the typical vascular ring is interrupted by gaps other than those due to the leaf-traces, and it may therefore be termed polystelic. In addition there are two or three small accessory steles lying within the vascular ring. Throughout the internode the course of these internal steles is quite free from the vascular ring, but at each node one of them approaches the free margin of the leaf-gap, and completely fuses with it, separating off again after the leaf-gap has become filled up.

Pteris elata, var. *Karsteniana*, has a typically solenostelic vascular ring, and also possesses internal accessory steles, which behave in a manner quite similar to those of *Dicksonia rubiginosa*; but they are relatively larger, and frequently they all fuse up together so as to form a second, inner, completely closed vascular ring.

It is suggested that the several internal steles and vascular rings that occur in the *Saccolomas* and in *Matonia pectinata* are also of the same origin and nature as those described above.

The relation of the internal accessory steles in certain Cyatheas to those of the above-mentioned Ferns is also discussed.

D. T. GWYNNE-VAUGHAN.

GLASGOW.

ON THE ANATOMY OF DANAEA AND OTHER MARATTIACEAE¹.—Various species of the Marattiaceae were studied for the comparative anatomy of the adult structure, and *Danaea simplicifolia*, Rudge, for the development of the vascular system.

I. Development of the vascular system of *Danaea simplicifolia*.

The primary vascular axis is a simple concentric stele. The xylem

¹ Abstract of paper read before the Botanical Section of the British Association, Glasgow, September, 1901.

consists of a central mass of small scalariform tracheids, without any conjunctive parenchyma. The phloem consists of a layer of small sieve-tubes separated from the xylem by a layer of parenchyma. The pericycle may be absent or only imperfectly represented. There is a definite endodermis, but the constituent cells are not clearly always the innermost ones of the extrastelar parenchyma.

When the cotyledon-trace is about to be given off, the xylem of this vascular axis, or 'protostele,' is separated into more or less unequal portions by a layer of parenchyma. The parenchyma increases in amount, and ultimately the cotyledon-trace is separated from the central stele. The cotyledon-trace is collateral. The next few leaf-traces are given off in the same manner, and are likewise collateral. The stele resumes its simple 'protostelic' appearance. Cauline roots occur, but not regularly.

As further leaf-traces depart from, and root-traces join the vascular axis, the primitive structure is gradually modified, and it may become more or less crescentic, forming an incomplete, or even complete, gamostelic ring. The spaces left by the departure of the leaf-traces now constitute leaf-gaps. The vascular tissue of this stage may be described as a 'siphonostele with leaf-gaps.'

The time of appearance of the first mucilage-canal varies. The earliest occurrence noted was after the third leaf-trace had been differentiated.

In one seedling a curious ligament of phloem was observed, which pursued an oblique course upwards and connected the two horns of a crescentic vascular mass. This strand of phloem interrupted the course of the central mucilage-canal.

At first the leaf-traces are simple and collateral; later they are simple and concentric; still later each trace divides into a pair of strands as it recedes from the axis. At a higher level the leaf-trace consists of a pair of strands, each of which takes its departure separately.

A remarkable deviation in the early stages of development was shown by one seedling. A mass of parenchyma early made its appearance in the centre of the xylem, simulating a pith. Careful examination showed that this was due to abortion of the cotyledon and its trace, and exceptionally early preparation for the departure of the three succeeding leaf-traces.

2. Stele of the Marattiaceae.

The structure of the 'stele,' as seen in transverse section, is singularly uniform in essential histological details throughout the group. It may be said to be of the Fern type, but there is no endodermis (i.e. in the case of well-grown plants), and the pericycle is not characteristically present.

The protoxylem is usually endarch—at any rate in the frond—but it may be mesarch.

The protophloem is internal. This was first demonstrated in the steles of the stem by Miss Shove¹. It has since been found to be internal in the steles of the frond of two species of *Danaea* and of *Marattia alata*. There can be little doubt that the internal position of the protophloem is general for the steles of both stem and frond in this group of Ferns.

3. Apical growth.

All the fresh evidence obtained while studying the seedlings of *Danaea simplicifolia* is in favour of an initial group, consisting of a few cells, both in stem and root.

4. Roots.

Nothing new has been observed in the roots of the Marattiaceae. In the roots of *Danaea simplicifolia* there is what might be called a fibrous pith, which is early differentiated, even before the main mass of the xylem has begun to be lignified.

GEORGE BREBNER.

UNIVERSITY COLLEGE, BRISTOL.

THE ANATOMY AND DEVELOPMENT OF THE STEM IN THE PTERIDOPHYTA AND GYMNOSPERMS².—Comparatively little attention has been directed to the subject of the development of the stem. This research concerns itself chiefly with the development of the cauline fibro-vascular skeleton, since this appears to be most interesting from the phylogenetic and morphological standpoints. A study of numerous examples drawn from the main groups of the Pteridophyta and Gymnosperms has led to the conclusion that the polystelic type of Van Tieghem does not originate, as he states, by the repeated bifurcation of the epicotyledonary central

¹ Annals of Botany, vol. xiv, 1900, p. 497.

² Abstract, republished from the Proceedings of the Royal Society, vol. lxi, 1901.

cylinder; but that the latter becomes at first a concentric fibro-vascular tube (*Bündelrohr* of De Bary), with gaps for the branches alone, or with gaps for both leaves and branches.

The tubular nature of the central cylinder in the polystelic type may become subsequently disguised by the overlapping of the gaps and by the appearance of medullary strands, derived in all the cases investigated by the writer from the inner wall of the stelar tube. It seems better to describe these conditions as adelosiphonic instead of polystelic, since the latter term implies a misconception.

In the Osmundaceae the writer believes he has found evidence of the derivation of the medullated monostelic and astelic types from the siphonostelic condition with internal phloem by the degeneration of the latter.

Osmunda cinnamomea shows all stages between the polystelic and astelic conditions; *O. regalis* still retains occasionally a brown sclerenchymatous pith, while in *O. claytoniana* this phenomenon is quite absent. Similar examples of degeneracy are found among the Polypodiaceae. Potonié further believes that the so-called medullated monostelic central cylinder of the Gymnosperms is derived by degeneracy of the internal phloem from such types as *Medullosa*. The writer considers that there is good evidence for regarding the so-called medullated monostelic type of central cylinder as derived by specialization, accompanied by degeneracy, from the so-called polystelic type of Van Tieghem, and thus returns to the conception of the morphology of fibro-vascular strands set forth in De Bary's 'Comparative Anatomy.'

The study of the development of the fibro-vascular skeleton of the higher plants seems to lead to the conclusion that this is hardly less important phylogenetically than the osseous skeleton has proved to be in the case of vertebrated animals. Where the tubular central cylinder exists there are two main types, the phyllosiphonic, where foliar gaps are constantly present, and the cladophonic, where foliar gaps are equally constantly absent. The central cylinder of the Filicales, Gymnosperms, and Angiosperms belongs to the former type, and that of the Lycopodiales and Equisetales to the latter. These distinctions appear to be of special importance, on account of the absence of constant and far-reaching criteria of taxonomy among the vascular plants. They moreover agree closely with evidence drawn from other available sources.

The writer is of opinion that there are two great primitive stocks of vascular plants, the Lycopsidea and Pteropsida. The Lycopsidea include the Lycopodiales and Equisetales, and are palingenetically microphyllous and cladosiphonic. The Pteropsida include the Filicales and Phaenogams, which are primitively megaphyllous and phyllosiphonic.

EDWARD C. JEFFREY.

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Vol. XV. No. LVII. MARCH, 1901. Price 14s. (\$3.50).

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EDITED BY

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ASSISTED BY OTHER BOTANISTS

London

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CLARENDON PRESS DEPOSITORY, 116 HIGH STREET

1901

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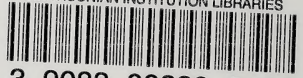
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